Genetic and systemic factors in knee osteoarthritis and its symptoms

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Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by any other person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Statement of Authorship

This thesis includes papers for which Feng Pan (FP) was not the sole author. FP was the first author in the research of each manuscript; however, he was assisted by the co-authors whose contributions are detailed below:

Chapter 4

Pan F, Khan H, Ding C, Winzenberg T, Martel-Pelletier J, Pelletier JP, Cicuttini F, Jones G. *Familial effects on structural changes relevant to knee osteoarthritis: a prospective cohort study*. Osteoarthritis Cartilage, 2015; 23(4):559-64.

FP was responsible for data collection, data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft, and completed manuscript revisions.

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Chapter 5

<u>Pan F</u>, Blizzard L, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *The interaction between weight and family history of total knee replacement with knee cartilage: a 10-year prospective study*. Osteoarthritis Cartilage, 2017; 25(2):227-233.

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Chapter 6

<u>Pan F</u>, Ding C, Winzenberg T, Khan H, Martel-Pelletier J, Pelletier JP, Cicuttini F, Jones G. *The offspring of people with a total knee replacement for severe primary knee osteoarthritis have a higher risk of worsening knee pain over 8 years*. Ann Rheum Dis, 2016; 75(2):368-373.

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Chapter 7

Pan F, Laslett L, Blizzard L, Cicuttini F, Winzenberg T, Ding C, Jones G. *Associations between fat mass and multi-site pain: a 5-year longitudinal study*. Arthritis Care Res (Hoboken), 2017; 69(4):509-516.

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Chapter 8

<u>Pan F</u>, Laslett L, Tian J, Winzenberg T, Cicuttini F, Ding C, Jones G. *Pain at sites outside the knee predicts knee cartilage volume loss in older adults: a prospective study.* Arthritis Care Res (Hoboken), 2017; 69(5):659-666.

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Abstract

Osteoarthritis (OA) is a multifactorial disease of the joints with a complex interplay between systemic factors, such as age, sex, genetic components, obesity and environmental factors (including smoking, diet, physical activity, joint injury and muscle function). Among those risk factors, genetic and modifiable factors (obesity) have been shown to have a crucial role in the development and progression of the disease on radiographs; however, how genetic factors and obesity influence the progression of early structures on magnetic resonance imaging (MRI) and its symptoms (pain) is not fully understood. This thesis aims to explore how these two factors separately or interactively are associated with important structural outcomes on MRI and pain.

Data from two longitudinal studies were utilised (the Offspring and TASOAC study). In the offspring study, 372 individuals (186 offspring having at least one parent with a total knee replacement (TKR) for severe primary knee OA and 186 controls) aged 26–61 years (mean age of 45 years) participated at baseline and were followed 2.3 and 10.2 years later. TASOAC study is a population-based study with 1099 older adults aged 50-80 years (mean age of 62 years) enrolled at baseline and followed approximately 2.6 and 5.1 years. Cartilage volume, cartilage defects, bone marrow lesions (BMLs), meniscal pathology and effusion were assessed by MRI. Radiographic OA was assessed by X-ray. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess knee pain. A self-reported questionnaire was used to assess pain at neck, back, hands, shoulders, hips, knees and feet. Fat mass was assessed using dual energy x-ray absorptiometry.

Data from the Offspring study was used to describe the associations of family history of knee OA with worsening knee pain and knee structural changes over 10 years. We found that offspring had an increased risk of worsening knee pain as compared to controls with no family history of knee OA, and this association was independent of structural factors. Also, offspring had an increased risk of worsening multiple knee structural abnormalities including cartilage defects, meniscal extrusion and tears but not BMLs.

The associations between weight and knee cartilage volume/defects over 10 years in offspring and in controls were also examined from the same population. Increasing body weight was deleteriously associated with medial tibiofemoral cartilage volume and presence of medial tibiofemoral cartilage defects in offspring. Similar associations were observed for lateral tibiofemoral cartilage volume and defects. However, there were no statistically significant associations between weight and cartilage volume or defects in controls.

The fourth study utilised data from the TASOAC study to explore the associations of fat mass, fat mass index (FMI) and body mass index (BMI) with multi-site pain (MSP), finding that fat mass was associated with MSP and pain at the hands, knees, hips and feet. Results were similar for FMI and BMI. The final study, in the same population, found that the presence of MSP independently predicts knee cartilage volume loss.

In conclusion, this series of studies suggest that both genetic and systemic factors (especially fat mass) may have an important role in early structural changes and pain in OA, and these two factors interact with each other to involve in the pathogenesis of OA.

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List of Publications

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Pan F, Blizzard L, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *The interaction between weight and family history of total knee replacement with knee cartilage: a 10-year prospective study*. Osteoarthritis Cartilage, 2017; 25(2):227-233.

Pan F, Ding C, Winzenberg T, Khan H, Martel-Pelletier J, Pelletier JP, Cicuttini F, Jones G. *The offspring of people with a total knee replacement for severe primary knee osteoarthritis have a higher risk of worsening knee pain over 8 years*. Ann Rheum Dis, 2016; 75(2):368-373.

Pan F, Laslett L, Blizzard L, Cicuttini F, Winzenberg T, Ding C, Jones G. *Associations between fat mass and multi-site pain: a 5-year longitudinal study*. Arthritis Care Res (Hoboken), 2017; 69(4):509-516.

Pan F, Laslett L, Tian J, Winzenberg T, Cicuttini F, Ding C, Jones G. *Pain at sites outside the knee predicts knee cartilage volume loss in older adults: a prospective study*. Arthritis Care Res (Hoboken), 2017; 69(5):659-666.

Manuscripts published during candidature, but external to thesis material:

Pan F, Ding C, Winzenberg T, Khan H, Martel-Pelletier J, Pelletier JP, Cicuttini F, Jones G. Response to: *'Does it make sense to investigate whether the offspring of people with a total knee replacement for severe primary knee osteoarthritis have a higher risk of worsening knee pain?' by Lei et al*. Ann Rheum Dis, 2015; 74(8):e45.

<u>Pan F</u>, Tian J, Winzenberg T, Ding C, Jones G. *Association between GDF5 rs143383 polymorphism and knee osteoarthritis: an updated meta-analysis based on 23,995 subjects.* BMC Musculoskelet Disord, 2014; 15:404.

Scientific Presentations and Awards

Oral presentations:

International conferences

2014 American College of Rheumatology (ACR) Annual Conference (Boston, US)

Pan F, Khan H, Ding C, Winzenberg T, Martel-Pelletier J, Pelletier JP, Cicuttini F, Jones G. *Does a Family History of Total Knee Replacement for Knee Osteoarthritis Influence Knee Pain and Structural Progression? a Prospective Longitudinal Cohort Study.*

2015 Annual European Congress of Rheumatology (EULAR) (Rome, Italy)

Pan F, Ding C, Laslett L, Tian J, Winzenberg T, Cicuttini F, Jones G. *Does a Pain at multiple sites outside the knee predicts knee cartilage volume loss: a prospective study in older adults?*

2015 American College of Rheumatology (ACR) Annual Conference (San Francisco, US)

Pan F, Laslett L, Cicuttini F, Thomson R, Winzenberg T, Ding C, Jones G. *Which factors explain multi-site pain caused by obesity: a 5-year follow-up study in older adults?*

2016 Annual European Congress of Rheumatology (EULAR) (London, UK)

Pan F, Aitken D, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *Does "pain elsewhere" influence the association between knee structural pathology and knee pain?*

Pan F, Squibb K, Thomson R, Winzenberg T, Zebaze R, Jones G. *Genetic effects on trabecular and cortical volumetric bone mineral densities and bone microstructure measured by HRpQCT.*

2016 American College of Rheumatology (ACR) Annual Conference (Washington, DC, US)

Pan F, Aitken D, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *Which Factors Associate with Localized Knee Pain and Generalized Pain: A 10-Year Longitudinal Study?*

2017 World Congress on Osteoarthritis (Las Vegas, US)

Pan F, Tian J, Aitken D, Cicuttini F, Winzenberg T, Ding C, Jones G. *Differentiating pain phenotypes in knee osteoarthritis: a 10-year prospective study.*

2017 Annual European Congress of Rheumatology (EULAR) (Madrid, Spain)

Pan F, Aitken D, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *Predictors and MRI-detected structural pathology with trajectories of knee pain severity: a 10.7-year prospective study.*

National conferences

2015 Population Health Congress (Hobart, Australia)

Pan F, Laslett L, Cicuttini F, Thomson R, Winzenberg T, Ding C, Jones G. *Pain at multiple sites outside the knee predicts knee cartilage volume loss: a prospective study.*

Invited Speaker

2016 Australian Sport Medicine State Conference (Hobart, Australia)

Pan F. *The Role of Genetic and Systemic Factors in Osteoarthritis-related Pain.*

Poster presentations:

International conferences

2016 Annual European Congress of Rheumatology (EULAR) (London, UK)

Pan F, Blizzard L, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *Does weight in the offspring of people with a total knee replacement for severe primary knee osteoarthritis have a more detrimental effect on knee cartilage and pain? a 10-year prospective study.* (Poster tour presentation)

2016 World Congress on Osteoarthritis (Amsterdam, Netherlands)

Pan F, Blizzard L, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *Does more weight in the offspring of people with a total knee replacement for severe primary knee osteoarthritis have a more detrimental effect on knee cartilage and pain? a 10-year prospective study.*

Pan F, Aitken D, Tian J, Cicuttini F, Winzenberg T, Ding C, Jones G. *Does pain at other sites influence the association between knee pathology and knee pain?*

2016 Annual European Congress of Rheumatology (EULAR) (Madrid, Spain)

Pan F, Tian J, Aitken D, Cicuttini F, Ding C, Jones G. *Characterizing and validating the phenotype of knee pain: a latent class analysis.* (Poster tour presentation)

National conferences

2015 Annual Scientific Meeting of Australia Rheumatology Association (ARA) (Adelaide, Australia)

Pan F, Laslett L, Cicuttini F, Thomson R, Winzenberg T, Ding C, Jones G. *Pain at multiple sites outside the knee predicts knee cartilage volume loss: a prospective study.*

2015 Australia New Zealand Bone & Mineral Society (ANZBMS) Annual Scientific Meeting (Hobart, Australia)

Pan F, Thomson R, Brown M, Leo P, Cicuttini F, Winzenberg T, Ding C, Jones G. *Genetic effects on trabecular and cortical volumetric bone mineral densities and bone microstructure.*

Pan F, Squibb K, Thomson R, Winzenberg T, Zebaze R, Jones G. *Genetic variants associated with bone mineral density in hip/spine are not related to osteophytes, bone marrow lesions, bone mineral density and area in subchondral bone.*

Local conferences

2014 Sharing Excellence in Research (SEiR), University of Tasmania, Conference (Hobart, Australia)

Pan F, Khan H, Ding C, Winzenberg T, Martel-Pelletier J, Pelletier JP, Cicuttini F, Jones G. *Does a family history of total knee replacement for knee osteoarthritis influence knee pain and structural progression? A prospective cohort study.*

2015 Sharing Excellence in Research (SEiR), University of Tasmania, Conference (Hobart, Australia)

Pan F, Ding C, Laslett L, Tian J, Winzenberg T, Cicuttini F, Jones G. *Pain at multiple sites outside the knee predicts knee cartilage volume loss: a prospective study in older adults.*

Awards

2013 Tasmania Graduate Research Scholarship for current PhD;

2014 Finalist of the Australian Society for Medical Research (ASMR) Postgraduate Student Competition;

2014 University of Tasmania Conference and Research Travel Funding ($2,000): to attend 2014 ACR Annual Conference;

2015 'Ten of the best' award, Menzies Institute for Medical Research ($1,000): for outstanding academic performance of staff and students over the preceding 12 months;

2015 Australasian Epidemiological Association Student Award ($500): to attend 2015 Population Health Congress;

2015 EULAR travel bursaries (amount to $2,000): to attend 2015 Annual European Congress of Rheumatology;

2015 Chinese Government Award for Outstanding Students Abroad ($8,000);

2016 EULAR travel bursaries (amount to $2,000): to attend 2016 Annual European Congress of Rheumatology;

2016 Arthritis Australia Fellowship 2017 ($50,000)**.** This is a competitive award offered to a junior investigator to further work in osteoarthritis. Only one fellowship is awarded annually;

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List of Abbreviations

ACR	American college of rheumatology
BMI	body mass index
BMLs	bone marrow lesions
CIs	confidence intervals
COMT	catechol-O-methyltransferase
CRP	C-reactive protein
CV	coefficient of variation
CWP	chronic widespread pain
dGEMRIC	delayed magnetic resonance imaging of cartilage
DMOAD	disease-modifying osteoarthritis drugs
DXA	dual-energy X-ray absorptiometry
FMI	fat mass index
FTO	fat mass and obesity-associated
GDF5	growth differentiation factor 5
GWAS	genome-wide association scans
ICCs	intraclass correlation coefficients
IL-6	interleukin-6
JSN	joint space narrowing
K&L	kellgren and lawrence
KOOS	Knee injury and Osteoarthritis Outcome Score
LSC	least significant criterion
MHC	major histocompatibility complex
MRI	magnetic resonance imaging

MSP	multi-site pain
NSAIDs	non-steroidal anti-inflammatory drugs
OA	osteoarthritis
OARSI	osteoarthritis research society international
OR	odds ratio
PCSK6	proprotein convertase subtilisin/kexin type 6
PET	positron emission tomography
QST	quantitative sensory testing
RCT	randomised controlled trials
ROA	radiographic osteoarthritis
RR	relative risk
SD	standard deviation
SNPs	single nucleotide polymorphisms
TASOAC	Tasmanian Older Adult Cohort
TGF-β1	transforming growth factor-β1
TJR	total joint replacement
TKR	total knee replacement
TNF-α	tumor necrosis factor-α
TRPV1	transient receptor potential cation channel subfamily V member 1
UK	United Kingdom
US	United States
WOMAC	Western Ontario and McMaster University Osteoarthritis Index
WORMS	whole-organ magnetic resonance imaging score

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Chapter 1: Introduction

1.1 Overview of osteoarthritis

Osteoarthritis (OA) is the most common form of joint disease worldwide and is a leading cause of pain and impaired function among working age and older adults, afflicting 9.6% of men and 18% of women aged more than 60 years [1]. It often affects hand and lower extremity joints such as the knee and hip, with an estimated lifetime risk for knee OA of being approximately 40% for men and 47% for women [2]. The proportion of any doctor-diagnosed OA is estimated to increase from 26.6% in 2012 to 29.5% by the year of 2032 in the population aged ≥45 with increasing of ageing population, life expectancy and prevalence of obesity [3]. By 2050, it is projected that prevalence of OA will increase to 3.14 million Australians or 10.7% of the population [4].

OA used to be considered as a disorder of the articular cartilage, but it is widely recognised that the condition involves the entire joint involving the loss of articular cartilage, subchondral bone remodelling, the formation of osteophytes, the development of bone marrow lesions (BMLs), thickening of the joint capsule, ligamentous laxity, weakening of muscle and meniscal tears and extrusion [5-7]. The common symptoms of OA are joint pain related to use, joint stiffness of short-lasting inactivity, restricted movement and cracking of joints [8].

1.2 The impact of OA on health care and its burden

OA is a highly burdensome condition leading to a large societal and economic burden, which is largely attributed to the influences of disability, comorbid disease and the expenditure of treatment [9]. It is reported that the cost of care in developed countries accounted for about 1.0% to 2.5% of gross domestic product, and the trend of costs was increasing [10]. In United States (US), the total medical expenditures for arthritis and other rheumatic conditions increased from $233.5 billion in 1997 to $321.8 billion in 2003, most of which were the costs specific to OA [9]. In Australia, the fourth largest direct health expenditures ($4.0 billion) in 2004-05 were for arthritis and other rheumatic conditions in which OA accounted for nearly one third of total expenditures, mainly due to knee and hip replacements [11]. From 2000-01 to 2004-05, the amount spent on admitted patient services for OA increased by 82% [11]. With increasing rate of replacement operations, the health expenditures for OA are projected to have a strong upward trend. For instance, the number of knee replacement in US and Scandinavia more than doubled between 1999 and 2007-08 [3]. In addition to health-care related costs of OA, it is very challenging to estimate OA-related indirect costs, such as losses of productivity associated with reduced employment rate, absenteeism, presenteeism and lost retirement income. Although OA is not a life-threatening condition to individuals, it does have a significant impact on individuals' physical and psychological outcomes related to pain, impairment of activity, and reduced quality of life [12].

1.3 The epidemiology of knee OA

The knee joint is the most frequently affected site with prevalence increasing with age, and it is more common in women than in men [13]. It was estimated that the global prevalence of radiographically confirmed symptomatic knee OA was 3.8% in 2010, and its prevalence arrived at peak at around 50 years old, as shown in Figure 1-1 [13]. In US and European populations, the prevalence of knee OA with severe radiographic changes is around 1.0% of people aged 25-34 years, but increases to about 50% in those aged 75 years and above. The Framingham study reported the prevalence of radiographic knee OA increased from 19.2% in those aged over 45 years to 43.7% in those over 80 years [14]. Furthermore, there are geographical variations in the prevalence of knee OA with highest observed in the Asia Pacific high-income region [13]. Together with hip OA, knee OA has been ranked as the 11th highest contributor of the 291 conditions to global disability. Given the high prevalence of knee OA and its crucial role in independent ambulation leading to considerable disability preventing knee OA patients participating in society and independent living, this thesis mainly focuses on knee OA.

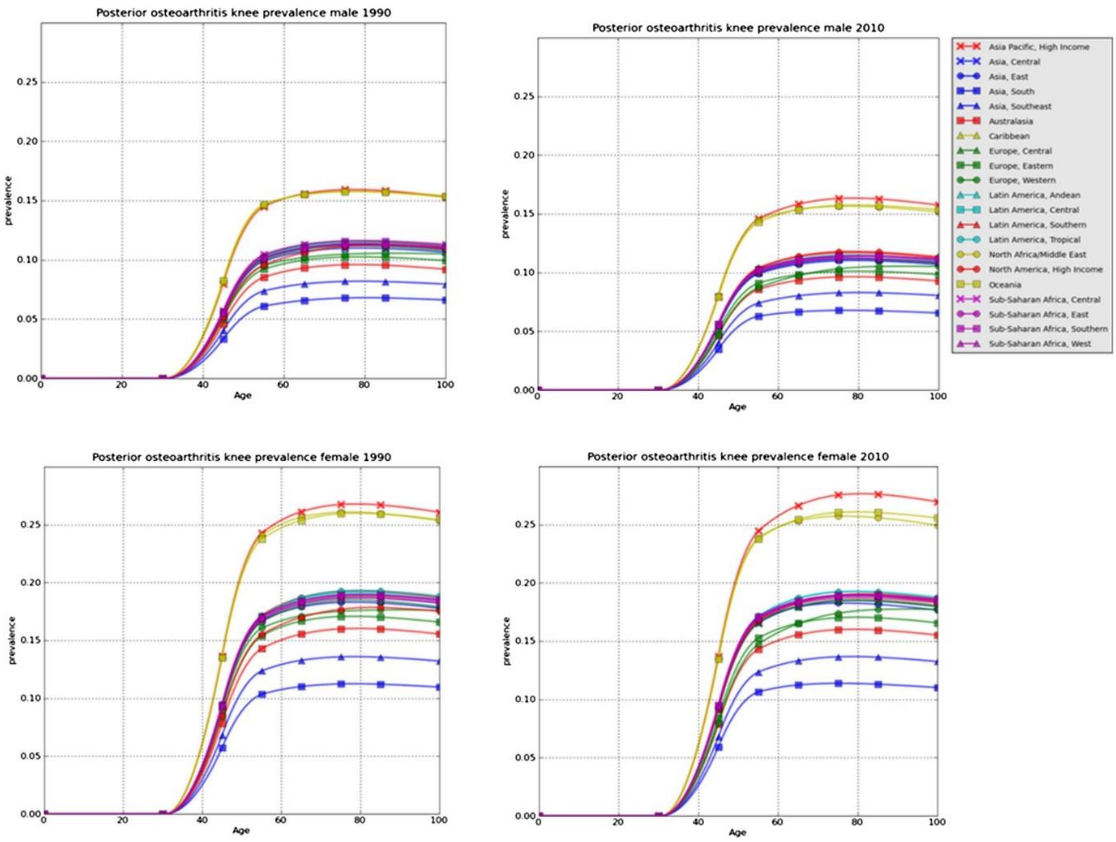

Figure 1-1 1990 and 2010 prevalence of knee osteoarthritis by age, sex, year and region, Global Burden of Disease 2010 study [13].

1.4 Diagnosis of OA

1.4.1 Radiographic OA

OA is traditionally diagnosed by conventional plain film radiography, with features of narrowing of the joint space width, osteophytes formation, the development of subchondral sclerosis and cysts [15]. Although radiography is often criticized as lack of sensitivity to detecting early stage of disease, lack of specificity to differentiating structures of cartilage thickness and meniscus behind joint space width that is subject to influence of joint positioning [16], it is still the current standard for evaluating joint structures in randomised controlled trials (RCT) of potential disease-modifying osteoarthritis drugs (DMOAD) by the regulatory agencies because it is cheap and readily available [17]. Lots of attempts have been made to accurately define and grade radiographic OA, two scoring systems have been widely using--Kellgren and Lawrence (K&L) score [18] and the Osteoarthritis Research Society International (OARSI) radiographic altas [19]. K/L system scores OA severity of joint space narrowing (JSN) and osteophytes on a scale from 0 to 4 with a cut-off of 2 or greater defining definite radiographic OA (Table 1-1); whereas the OARSI altas system scores JSN and osteophytes separately and gives a distinct score (Table 1-2).

Table 1-1 Definition of the Kellgren-Lawrence radiographic grades (knee)

Grade	Description
0: No osteoarthritis	No features of osteoarthritis
1: Doubtful	Doubtful narrowing of joint space and possible osteophytic lipping
2: Mild	Definite osteophytes and possible narrowing of joint space
3: Moderate	Moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
4: Severe	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends

Table 1-2 Osteoarthritis Research Society International altas of radiographic features of OA (knee)

Site and feature	Description			
Knee--tibiofemoral				
Marginal osteophytes				
Medial femoral condyle	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Medial tibial plateau	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Lateral femoral condyle	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Lateral tibial plateau	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Joint space narrowing				
Medial compartment	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Lateral compartment	0 (normal)	1 (mild)	2 (moderate)	3 (severe)
Other				
Medial tibial attrition	0 (absent)		1 (present)	
Medial tibial sclerosis	0 (absent)		1 (present)	
Lateral femoral sclerosis	0 (absent)		1 (present)	

1.4.2 Clinical OA

OA also can be defined clinically by features in the medical history and on physical examination. In addition to other clinical features, the presence of joint pain is essential to define clinical OA. Currently, the American College of Rheumatology (ACR) criteria are the well-recognised standards in the diagnosis of clinical knee [20], hip [21] and hand OA [22].

1.5 Magnetic Resonance Imaging (MRI) in OA

Although MRI is not routinely utilised in clinical assessment due to high cost of examination, it has become a key imaging tool in OA research considering its potential to be more sensitive to detecting earlier disease and structures changes which cannot be detected on radiographs, and the capacity to visualise joint structures changes in three-dimensional fashion such as cartilage, menisci, BMLs, synovitis and effusion [23]. As shown in Figure 1-2, MRI can detect structural abnormities earlier than radiography.

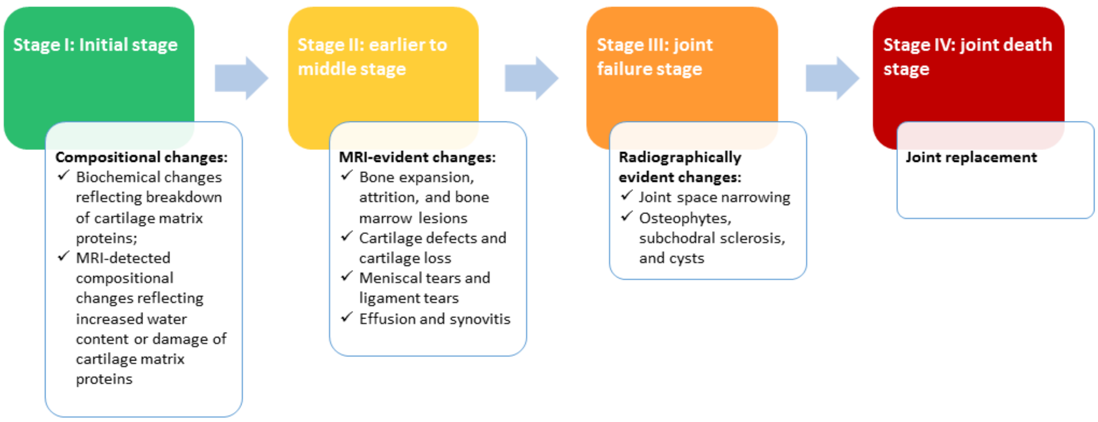

Figure 1-2 Osteoarthritis at different stages.

Knee cartilage is the most common joint structure measured on MRI. Cartilage damage remains the major focus in assessing the development and progression of knee OA [24]. The validity, accuracy, reliability and sensitivity of MRI using semiquantitative as well as quantitative scoring methods in detecting cartilage damage have been well demonstrated [25, 26]. Cartilage volume loss and defects have shown their clinical relevance through predicting knee replacements [17, 27-29], but they do not have consistent evidence showing their relationships with symptoms (knee pain), possibly because of aneural and avascular articular cartilage [30].

The presence of BMLs is an important feature of knee OA. They can be present in those with both early asymptomatic [31, 32] and late-stage knee OA [33, 34]. Histologic changes of BMLs consist of bone marrow necrosis (11%), abnormal trabeculae (8%), bone marrow fibrosis (4%), bone marrow edema (4%), and bone marrow bleeding (2%). BMLs can be evaluated using fluid-sensitive fast spin echo sequences with fat suppression (T2-weighted), scored using semiquantitative techniques such as Whole-Organ Magnetic Resonance Imaging Score (WORMS) or a fully automated method [35, 36]. It has been reported in previous studies that BMLs size and score fluctuate in a short time with the possibility of complete resolution [35, 37, 38]. BMLs in early or advanced disease have been found to be associated with cartilage volume loss, progression of cartilage defects in a both short and long time-frame [31, 39-41], and knee replacement [17, 29, 42-45]. More recently, prior studies have shown the relation of BMLs to severity [46], incidence [47] and fluctuation of pain [48]. Despite some conflicting results, the evidence supports a relatively consistent relation of BMLs to pain [47].

Meniscal damage commonly seen in knee OA is often a result of the increased biomechanical loading due to knee malalignment, obesity or injury [49]. Meniscal pathology can be viewed and scored by MRI using either quantitative or semiquantitative methods [50]. There are two main types of meniscal pathology including meniscal tears and extrusion in the semiquantitative method. Meniscal pathology is considered an important structural change in knee OA, with significant associations between meniscal tears and cartilage loss [50-52], between meniscal extrusion and cartilage defects [50-52], and even a predictive role of meniscal pathology with knee replacement [17, 29, 42]. However, to date, there is no consistent evidence to support their relations to the presence of knee pain [47].

Synovitis-effusion is frequently present in knee OA. It is reported that nearly 90% of referred knee OA patients have synovitis [53]. Synovitis-effusion can be assessed on MRI using quantitative or semiquantitative methods [54]. Currently, non-contrast-enhanced MRI is often used to measure synovitis-effusion in the research and clinical trials due to cost and the potential side-effects of gadolinium, although contrast enhancement has an ability to differentiate the thickened and inflamed synovium from synovial fluid [17]. Synovitis-effusion assessed on non-contrast-enhanced MRI has been shown to associate with increased cartilage defects, BMLs and cartilage volume loss [55, 56]. These features also correlate with clinical prognosis and predict knee replacement [29]. A moderate association of synovitis-effusion with pain severity and pain fluctuation has been consistent in previous studies [57, 58]. So far, a preliminary definition in the use of MRI for assessing OA status has been developed, but it still needs further validation and testing in the clinical and research setting [59].

In most cases, structural abnormalities detected on MRI are present several years before the development of radiographic disease, so targeting these early structural changes may prevent OA progression. Cartilage loss is currently considered a hallmark of OA, identifying risk factors in those at high risk of developing OA may be of particular relevance to OA prevention [60]. With the development of new MRI techniques that can identify the compositional changes within these structures, such as delayed magnetic resonance imaging of cartilage (dGEMRIC) [61], T2 mapping [62] and T1Rho [63], these may advance our outstanding of the role of structural abnormalities in the pathogenesis of OA.

1.6 Risk factors for OA

It has been recognised that knee OA appears to be determined by a complex interplay between systemic factors, such as age, sex, genetic components, obesity and environmental factors determined by smoking, diet, physical activity, joint injury and muscle function, although the OA's aetiology is not fully understood [14]. Table 1-3 summarises the joint-specific risk factors. Environmental factors increasing risk of developing OA are mostly related to joint biomechanics in nature and adversely have an influence on the joint forces [15, 50]. However, there is increasing evidence that most people who have abnormal joint biomechanics do not develop OA, suggesting individual susceptibility may be partly determined by systemic factors through predisposing individuals to joint injury, causing direct damage to joint tissues, or affecting function of repair in damaged joint tissue [14]. Therefore, targeting persons with systemic risk factors may allow for the early prevention and diagnosis of OA, such as family history of OA and obesity which are discussed in great details below and examined in **Chapter 4 and Chapter 5**.

Table 1-3 Risk factors for development of osteoarthritis

Risk Factor	Hip OA	Knee OA	Hand OA
Obesity	(+)	+	(+)
Age	+	+	+
Female sex		+	+
Genetics	+	+	+
Smoking			
Physical activity	(−)	(−)	
joint injury		+	
Muscle			
Grip Strength			+
Quadriceps		(−)	

+, good evidence increases risk; (+), weak evidence increases risk; blank, inconsistent or no evidence of increased risk; (−), weak evidence of protective effect; −, good evidence of protective effect.

1.7 OA-related pain

Pain is a subjective and complex phenomenon in nature, with the influence of bio-psychological and social factors [64], which is reflected by definition of pain from the International Association for the Study of Pain as “Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” [65].

Musculoskeletal pain is very common in general population in western countries affecting approximately 13.5-47% of people [66]. Due to high impact on disability, it is a major public health burden [67]. It has been estimated that 30% of American adults are afflicted by musculoskeletal pain at any one time [68], and 16% of UK adults have pain more than three joints which last more than one week during one month [69]. The causes of musculoskeletal pain encompass a spectrum conditions, but OA is the most common cause of pain. It is reported that 20% of musculoskeletal pain is attributable to OA in Europe [70]. This proportion increases markedly with age, with one study showing that 81% and 78% of persons with hand pain and knee pain had a definite radiographic hand OA and knee OA [71].

1.7.1 Single-site pain (knee pain)

Knee pain is the prominent symptom of knee OA, which drives individuals to seek healthcare, contributes to restrictions in function and reduced quality of life [72]. Also, knee pain is main reason for people seeking for joint replacement [72]. According to two pain surveys [73, 74], the prevalence of knee pain in older adults was estimated about 25%. Furthermore, a United Kingdom (UK) study also reported a prevalence of 21% and 35% in men and women aged 45 or above for persistent knee pain lasting for at least a week in the previous month [69]. Although some other studies estimated a relative lower prevalence of knee pain, these differences can be explained by the variation of cases definition, composition of studied population and pain questionnaire utilised [75].

1.7.2 Multi-site pain

In pain research, a new concept of ‘multi-site’ or ‘multiple site’ pain has been proposed, which means pain occurring at more than one site. Currently, there is no clear definition for multi-site pain (MSP), one definition for chronic widespread pain (CWP) based on the diagnostic criteria for fibromyalgia was developed by the ACR in 1990 [76]. It requires the presence of pain in the axial skeleton, on the left and right, above and below the waist for at least three months. However, in clinical reality, there is no cut-off point available in defining MSP [77]. Using definitions of widespread pain from ACR often excludes the majority with MSP [78, 79]. It has been suggested that counting the number of painful sites could be of particular importance and relevance to managing musculoskeletal pain [77].

There is much evidence showing that people having pain at one site are more likely to report pain at other sites concurrently [80-85]. In a study comprising 12,410 adults from 18 countries, 41% of people reported pain more than two sites out of six anatomical regions [84]. A population-based study in UK showed that three quarters of people had pain at two or more sites out of 13 body sites [79]. Two-thirds of people having pain in at least two sites of six body sites in the last 12 months were reported in a Greek population [86]. Compared to single-site pain, MSP is associated with poorer level of physical and psychological health, worse health-related quality of life, and more severe depressive symptoms in both cross-sectional and longitudinal studies [87-90]. Given more prevalent MSP and more serious impact on health than single site pain, pain at one site should not be considered in isolation, but assessment of pain at other sites should be stressed. Therefore, the relationship between MSP and cartilage volume loss are investigated in **Chapter 8**.

1.7.3 Measure of pain in OA

In current pain research, there are a number of questionnaires available to assess pain. The most common questionnaires include generic unidimensional pain questionnaires (Visual Analog Scale and Numeric Rating Scale), generic multidimensional pain questionnaires (Short-form McGill Pain Questionnaire, Chronic Pain Grade Scale, and Short Form-36 Bodily Pain Scale), and an arthritis-specific pain questionnaire (Measure of Intermittent and Constant Osteoarthritis Pain, the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the Knee injury and Osteoarthritis Outcome Score (KOOS)) [91, 92]. Some other additional questionnaires have been developed and validated with consideration of the multidimensional nature of pain, such as the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials [93] and Patient Reported Outcomes Measurement Information System [94]. Despite that these questionnaires have been widely used for pain assessments, each questionnaire has its own strengths and weakness. As a result, it is impossible that complex pain experience with variation in pain intensity, frequency, pattern and quality can be adequately captured by a single existing questionnaire. Furthermore, differences in use of questionnaires in OA research may result in the variation in reported pain prevalence. Question about presence of pain over a specified period (e.g. “pain on most days of a month in the past year” vs “pain on most days of the past month”) of time may be prone to recall bias [95]. Also, simple pain questionnaires current studies have been using are insufficient to allow complex interactions to be ascertained, this might be one of possible explanations for poor correlations between structures and pain in OA. Other than pain intensity, pain questionnaire should keep with the acknowledgment of the multidimensional nature of pain with the consideration of physical functioning, socio-psychological functioning, as well as other domains such as fatigue, sleep, and cognition. However, to date, there is no pain questionnaire available in this kind. Pain questionnaire should be improved and refined in future.

1.7.4 Structural damage and OA-related pain

Pain in OA has been considered nociceptive pain, arising from stimulation of peripheral local tissue damage [96]. Cartilage, the primary site of OA pathology, is aneural and avascular, so cannot generate pain directly, raising the possibility that pain may come from other structures [96]. By contrast, subchondral bone, adjacent periosteum, synovial membrane, periarticular ligaments and joint capsule are richly innervated with the nerve fibres transmitting peripheral input to spinal cord [8]. However, imaging studies have widely reported a significant discordance between radiographic severity of OA and knee pain [72]. A systemic review of literature concluded that 15-76% of patients with knee pain had radiographic OA, and 15-81% of patients with radiographic OA had knee pain [97]. This discordance is often explained by the ability to discern underlying pathologies contributing to pain [57]. As stated above, in light of the ability of visualising detailed structures on MRI, some studies have examined the relationships between structures on MRI and knee pain, and reported inconsistent results [57]. This is supported by a recent literature review concluding only thirteen of twenty-one studies reporting statistically significant associations of MRI findings in OA and symptoms [98]. Table 1-4 summaries the associations between structures detected on MRI and knee pain. Overall, the levels of evidence between structural features and pain are limited or conflicting, except for

BMLs and effusion-synovitis which appear to have a moderate levels of evidence supporting their relation to OA-related pain [72]. Lack of strong evidence of the associations between structures detected on MRI scans or radiographs suggests that pain may be mediated by other factors, such as genetic components, and there may be a central component to pain [99].

Table 1-4 Associations between knee structural factors detected by MRI and pain

Structures	Evidence				
	No	**Conflicting**	**Limited**	**Moderate**	**Strong**
Cartilage defects		+			
Meniscal pathology		+			
Bone marrow lesions				+	
Bone attrition		+			
Osteophytes			+		
Effusion-synovitis				+	
Ligament tear			+		
Tibial bone size	+				

The levels of evidence were using best evidence synthesis based on the guidelines on systematic review of the Cochrane Collaboration Back Review Group [100].

1.7.5 Pain mechanisms in OA

Various studies have also shown pain in OA is neuropathic, reflecting that the potential mechanisms of neuropathic pain is a consequence of the interrelation of peripheral and central sensitisation mechanisms [101-103]. Joint injury and/or inflammation lead to the release of mediators into the joint which sensitize primary afferent nerves with a reduction in threshold and an amplification of responsiveness to suprathreshold stimuli of peripheral nociceptors (peripheral sensitisation) [104-106]. As such, exaggerated responses to noxious mechanical stimuli (primary hyperalgesia) can be evoked, and normally innocuous joint movement can evoke a painful response (allodynia) [72]. Increased peripheral neuronal activity further confers the alteration in pain processing by central nervous system (central sensitisation) including more responsive to peripheral input, an expansion of receptive filed of dorsal horn neurons as well as brain activation, sensitisation and modification [99, 107]. Studies using quantitative sensory testing (QST) analyses and functional MRI have confirmed central sensitisation in OA [103, 107]. The presence of central sensitisation in OA may be predictive of more severe, longer duration and larger area pain which cannot be treated by conventional analgesics [102] and is a possible explanation for pain occurring at multiple sites.

1.7.6 Risk factors for OA-related pain

Pain is a very complex process affected by multiple interactive pathways including genetic, environmental, socio-economic and psychological factors [108]. Risk factors, such as environmental and psychosocial factors for single-site pain, have been extensively investigated in previous epidemiology studies, although the potential mechanisms of these factors contributing to pain are not yet well understood. At present, genetic contribution to OA-related pain is far less to know as comparing to genetics in OA. Furthermore, most of previous studies have exclusively focused on single-site pain and thought that risk factors identified is exclusive to each pain site, leading to uncertainties as to whether risk factors for MSP are different from single-site pain. Due to the importance of genetic factors and obesity in OA, it is possible that understanding these two factors in OA-related pain is also relevant to clinical practice in the diagnosis and treatment of OA. Therefore, this thesis is focusing genetic factors in OA-related pain (**Chapter 6**) and modifiable factors (obesity) in MSP (**Chapter 7**).

1.8 Treatment and management of OA and pain

Increased understanding of the pathogenesis of OA and its symptom (pain) allows the ability of identification of ‘at risk’ patients, diagnosis of early OA and evaluation of the efficacy of treatment within a short period. There are some new therapeutic interventions proposed and conducted in clinical trials with several drugs as disease modifying agents in OA, such as chondroitin and glucosamine; however, no therapeutic interventions to modify the structures and improve symptom concurrently have been approved so far [15]. Currently, therapeutic interventions for OA are palliative and primarily focus on alleviating pain [2]; however, treatment for the management of OA pain is problematic and mainly targets peripheral joint and peripheral nervous system. Non-steroidal anti-inflammatory drugs (NSAIDs) have been a mainstay treatment for OA pain [109], but the efficacy of these has been proven to be only moderate with more than 75% of symptomatic OA patients reporting need for additional symptomatic treatment [110]. Intra-articular glucocorticoid injections and joint replacement surgery also play a key part in the management of OA, both targeting peripheral mechanism of pain [111, 112]. Failure to relieve OA pain through these treatments is frequently seen in the clinical settings; for instance, there are approximately 7-23% and 10-34% of patients having long-term pain after hip and knee replacement surgery [113, 114], suggesting that treatments targeting peripheral mechanisms are insufficient for those patients. Hence, for those patients, targeting central pain processing may be more beneficial. An increasing number of drugs with central actions are also under investigation, such as duloxetine which has been approved by US food and drugs administration for the treatment of musculoskeletal pain [115].

Instead, modifiable risk factors (primarily obesity) have been a focus in the treatment and management of OA and pain especially when there is absent of pharmacologic agents that can modify disease. Good evidence is that weight loss in obese patients can reduce the risk of the development of symptomatic OA [116] and improve symptoms in OA patients [117]. Despite lack of radiographic structural modification through weight loss, it has been shown that weight loss has structure-modifying effects for obese individuals in morphological and physiological MRI [118] and significantly reduces low-grade systemic inflammation [119]. A combination of exercise and diet is recommended for weight management [120]. Benefits of exercise are evident with increase in muscle strength and aerobic capacity, [121], and with cardiovascular health and all-cause mortality [15], but the effects of exercise need to further elucidated. Some of other therapeutic options are available to help modify joint forces including knee braces, orthotics, patella taping and knee osteotomies [122]; however, in general, there is limited evidence for the effectiveness of these therapeutic interventions.

1.9 Genetic factors in OA

OA has been shown to be affected by a considerable underlying hereditary component, although it was considered a disease of age-related wear-and-tear on the cartilage of the affected joint [123]. The initial search for genetic components of OA started from observational studies -- twin pair, sibling risk and segregation studies which found an increased risk of the development of OA in the relatives of patients with Heberden's nodes [124] or hip OA [125], and the siblings of prohands undergoing total joint authroplasty [126]. Heritability estimated from previous studies has shown that 39–65% of the risk of developing OA can be explained by genetic components, which appears to be stronger in hip and hand OA than in knee OA, and varies depending on gender and severity of the conditions [127, 128]. This early evidence stimulated a considerable genetic search for genetic loci responsible for the susceptibility to OA. In 1990, Prockop *et al.* [129, 130] reported the first gene (COL2A1), which encodes for the alpha 1 polypetide chain of type II collagen, the principal collagenous component of articular cartilage, and that the mutation of this gene gives rise to impaired the matrix and premature degeneration of the cartilage.

In the past few decades, a number of candidate-gene studies have been conducted with few positive results replicated in the different populations [131]. Most of studies reported the false positive results possibly because of small sample sizes and lack of adjusted P values in reporting of significant results. Currently, there is one exception in candidate-gene studies in which rs143383 in the growth differentiation factor 5 (GDF5) gene showed a robust and significant association with OA across different populations with a genome-wide significance ($P<5\times10^{-8}$) [132]. With the advent of high throughput single nucleotide polymorphisms (SNPs) genotyping technology, it makes genome-wide association scans (GWAS) possible with hundreds of thousands of SNPs concurrently tested for association with disease. Currently, there are multiple loci identified from GWAS contributing to the susceptibility to the development OA

[133]. Table 1-5 lists the established loci with genome-wide significance in OA research. Although these studies further provided firm evidence of genetic component in OA, there is no single loci individually and substantially conferring increased risk of developing OA as comparing to autoimmune rheumatic diseases in which a significant influence of the major histocompatibility complex (MHC) region is involved in its susceptibility [134]. As shown in Table 1-5, the majority of odds ratios were less than 1.2, suggesting that a small effect of each individual allele determines the susceptibility to OA. Furthermore, significant joint-specific effects of genetic components were observed. Few loci have been found to have associations in both European and Asian populations with distinct ethnic differences in genetics, but the reason for these differences are still not clear. One of limitations of GWAS is that common variants, defined as frequencies >5% of the population are best assessed with limited ability to detect rare variants [128]. Another limitation is stringent statistical significance threshold; this therefore needs large sample sizes or very large genetic effects [128].

OA is a highly heterogeneous disease with variable clinical features. At present, there is no consensus on the definition of OA cases in current genetic studies; some centres used clinical or radiographic definitions whereas others used total joint replacement (TJR). These definitions cannot consider early changes of the disease in which different determinants may be involved, and thus further complicating the search for susceptibility alleles [123]. Therefore, standardised OA phenotypes are urgently needed in future genetic studies. More subtle structural changes visualised on MRI is increasing our understanding of the disease, so a possible solution is that using early structural abnormalities on MRI as phenotypes of OA may enable study of the genetic influences on OA better when no consensus definition of OA is available currently. Although evidence of a genetic predisposition to OA is ample, far fewer have been identified with OA progression. Genetic factors in the progression of early structural changes on MRI are examined in the **Chapter 4**. It is likely that genetic factors related to the onset of the disease may also promote its progression; however, there is sparse evidence to support these relationships.

Table 1-5 Established loci with genome-wide significance in OA research (from Panoutsopoulou K *et al.* [133])

SNP	Nearest* gene(s)	EA	EAF	OR, 95% CI	p Value	Site	Sex	Ethnic group	Source
rs143383†	GDF5	T	0.74	1.79, 1.53 to 2.09	2×10^{-13}	Hip	Both	Asian	[135]
rs143383†	GDF5	T	NA	1.16, 1.11 to 1.22	8.3×10^{-09}	Knee	Both	European	[132]
rs7639618	DVWA	G	0.63	1.43, 1.28 to 1.59	7.3×10^{-11}	Knee	Both	Asian	[136]
rs7775228‡	HLA-DQB1	T	0.62	1.34, 1.21 to 1.49	2.4×10^{-08}	Knee	Both	Asian	[137]
rs10947262‡	BTNL2	C	0.58	1.31, 1.20 to 1.44	5.1×10^{-09}	Knee	Both	Asian and European	[137]
rs3815148 §	COG5 ¶	C	0.23	1.14, 1.09 to 1.19	8×10^{-08}	Knee and hand	Both	European	[138]
rs4730250 §	DUS4L ¶	G	0.17	1.17, 1.11 to 1.24	9.2×10^{-9}	Knee	Both	European	[139]
rs11842874	MCF2L	A	0.93	1.17, 1.11 to 1.23	2.1×10^{-08}	Knee and hip	Both	European	[140]
rs6976**	GLT8D1††	T	0.37	1.12, 1.08 to 1.16	7.2×10^{-11}	Hip and knee	Both	European	[141]
rs11177**	GNL3††	A	0.38	1.12, 1.08 to 1.16	1.3×10^{-10}	Hip and knee	Both	European	[141]
rs4836732	ASTN2	C	0.47	1.20, 1.13 to 1.27	6.1×10^{-10}	Hip	Females	European	[141]
rs9350591	FILIP1; SENP6	T	0.11	1.18, 1.12 to 1.25	2.4×10^{-09}	Hip	Both	European	[141]
rs10492367	KLHDC5; PTHLH	T	0.19	1.14, 1.09 to 1.20	1.5×10^{-08}	Hip	Both	European	[141]
rs835487	CHST11	G	0.34	1.13, 1.09 to 1.18	1.6×10^{-08}	Hip	Both	European	[141]
rs12107036	TP63	G	0.52	1.21, 1.13 to 1.29	6.7×10^{-08}	Knee	Females	European	[141]
rs8044769‡‡	FTO	C	0.5	1.11, 1.07 to 1.15	6.9×10^{-08}	Hip and knee	Females	European	[141]
rs10948172	SUPT3H; CDC5L	G	0.29	1.14, 1.09 to 1.20	7.9×10^{-08}	Hip and knee	Males	European	[141]
rs6094710	NCOA3	A	0.04	1.28, 1.18 to 1.39	7.9×10^{-9}	Hip	Both	European	[142]
rs12982744	DOT1L	C	NA	1.17, 1.11 to 1.23	7.8×10^{-9}	Hip	Males	European	[143]

*Nearest gene(s) only shown.
†Summary statistics of the same SNP in separate studies in Asians and Europeans, respectively.
‡SNPs in strong linkage disequilibrium.
§SNPS in strong linkage disequilibrium.
¶chr7q22 locus encompasses more genes than shown here, for full details see Kerkhof *et al*. [138] and Day-Williams *et al*.[140]
**SNPs in strong linkage disequilibrium.
††chr3p21.1 locus encompasses more genes than shown here, for full details see arcOGEN Consortium.[141]
‡‡This signal was attenuated after BMI adjustment, suggesting that the FTO locus exerts its effect on OA through obesity.
BMI, body mass index; EA, Effect allele; EAF, effect allele frequency; OA, osteoarthritis; SNP, single nucleotide polymorphism.

1.10 Obesity/inflammation in OA

Obesity has become a serious public health issue worldwide over the past several decades. It affects all population and all age groups, resulting in extensive morbidity and mortality [144]. In Australia, the prevalence of overweight and obesity has been steadily rising for the past 30 years. A survey conducted in 2011-2012 reported that around 60% of Australia adults were classified as overweight or obese, more than 25% of whom were obese [145]. This upward trend imposes substantial obesity-related chronic diseases, including OA, and economic burdens.

The association between obesity and OA has been well-established in prior cross-sectional and longitudinal studies [146], with relatively stronger associations with knee OA than with hip OA [147]. Being overweight or obese is not only associated with the onset of OA [148], but also with an increased risk of OA progression [2]. A recent meta-analysis including 25 cohort studies by Silverwood *et al*. [139] showed a 2.1-fold increased risk of knee OA in overweight or obese individuals. A dose-response relationship between obesity and risk of knee OA has been demonstrated from a meta-analysis with 35% increased risk of knee OA being associated with every 5-unit increase in body mass index (BMI) [149]. Furthermore, studies examining associations between obesity and early structural changes on MRI have shown a deleterious effects of obesity or overweight on knee cartilage [150] and subchondral bone [151]. There were more consistent detrimental relationships between increasing weight or BMI and cartilage defects than that with cartilage volume [150]. Also, increasing BMI and weight have been found to be associated with increased prevalence of BMLs in both asymptomatic [31, 152, 153] and symptomatic populations [38]. Conversely, weight loss has shown a decreased risk for developing knee OA [154] and worsening early structures [150], and vice versa [155]. Taken together, the findings from previous studies suggest that increased weight or BMI may play a crucial role in early stage of the disease.

The mechanisms underlying the link between obesity and OA are complex. Joint loading has been considered an important role in this relationship; however, obesity not only confers an increased risk of weight-bearing joints, but also an increased risk of hand OA, indicating joint loading cannot completely explain its effects on OA, and raising the possibility that obesity may exert its effects on OA through metabolic and systemic inflammation [156, 157]. Indeed, adipokines, such as leptin, have been linked with the initiation of OA [158]. In animal model, obesity did not induce the development of OA in the absence of leptin. Consistently, an earlier study from our group found a reduced cartilage volume loss with elevated serum levels of leptin [159]. Our group also reported that serum levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are associated with knee cartilage volume loss [160], although the role of cytokines in the pathogenesis of OA remains to be elucidated [161, 162].

As stated above, OA is a heterogeneous condition as a result of a complex interaction between systemic and environmental factors. In some cases, the presence of genetic abnormality alone may not increase risk of OA, that is, it interacts with other environmental elements, such as obesity, contributing to risk of OA. It is possible that structural abnormalities caused by genetic factors creates an at risk environment, predisposing individuals to increased influence of environmental factors termed gene-environment interaction [163, 164]. Given strong genetic components and obesity underlying the pathogenesis of OA, understanding the interaction between genetics and obesity better will aid the prevention of OA. Their interactions are examined in the **Chapter 5**

1.11 Genetic factors in OA pain

Robust inter-individual differences in pain experience are often observed in the clinical sitting, raising the possibility that inter-individual variability in the experience of pain may be due to differences in pain sensitivity which is probably affected by underlying genetic factors [165]. Earlier twin and epidemiological studies have demonstrated that pain sensitivity per se is heritable [166, 167], although it has been suggested that a range of factors such as prior experience, expectation, and current mood modulate experience of pain and these factors themselves are genetically mediated [168-171]. The estimates of heritability from studies range from 9% to 60% for different pain traits [168, 169, 172]. The heritability of knee pain was estimated about 44% in a sib-pair study from our group [173].

With regard to these findings in prior studies, research has been trying to search for genes that might predispose individuals to development of chronic pain or experiencing greater pain sensitivity. There were two categories (linkage and candidate-gene studies) that the majority of studies have fallen into. A variety of genes identified have been shown to be tentatively associated with pain states [174, 175]. Like in other fields, there have been inconsistent associations in the replication across populations or across pain conditions. Other than the general reason for inconsistent results in genetic studies including sample size, pain definition, etc, the pain field struggles with the existence of complex pain phenotypes [174]. For example, pain conditions are quite heterogeneous even if one could identify genetic associations with specific pain conditions, such as low back pain, or there are a number of subcategories while investigating a broader category of clinical pain [165, 174]. Recent years have seen an explosion of GWAS in the identification of risk alleles; however, GWAS in human pain has lagged behind than in other fields, for reasons such as difficulties in undertaking the quantitative phenotyping of this subjective phenomena [176]. At present, there is only one adequately powered GWAS conducted for CWP [177].

Relative to numerous genomics studies in OA, only few studies have examined genes that regulate OA-related pain so far. Currently there are five genes identified with a possible association with pain in OA, as shown in Table 1-6. A common genetic variant of Val158Met in catechol-O-methyltransferase (COMT) gene which reduces the activity of the catecholamine degrading enzyme was identified to be associated with hip pain among those with hip OA [178]. Unfortunately, this SNP failed to be replicated for knee pain in an independent and adequately powered study [179]. Another gene SCN9A encoding the voltage gated Sodium Channel 1.7 (Nav1.7) that is essential for transmission of pain-related signals, was initially shown to associate with higher pain reports in a study with 578 OA patients [180], and held up in a larger cohort of replication study [181]. Some other candidate genes have been examined and have confirmed associations with OA-related pain, including transient receptor potential cation channel subfamily V member 1 (TRPV1), P2X7 and proprotein convertase subtilisin/kexin type 6 (PCSK6). No studies have investigated genetic factors in the pain evolution, and which genes can explain evolution in clinical and experimental pain responses. **Chapter 6** examines the effect of OA family history on worsening knee pain. Identifying genetic variants in a genetically enriched cohort may facilitate the stratification of population and our understanding of this extremely heterogeneous disorder.

Table 1-6 Genomics studies in osteoarthritis-related pain

Studies	SNP	Gene	Protein	Function of protein	Sample size (n)	Sex (%)	Ethnic group	Source
Initial study†								
	rs4680	COMT	Catechol-O-methyltransferase	Degradation of catecholamine neurotransmitters such as norepinephrine and dopamine	288 (radiographic hip OA)	59	Caucasian	[178]
					171 (female radiographic hip OA)	100	Caucasian	
	rs6746030	SCN9A	Voltage gated Sodium Channel 1.7 (Nav1.7)	Nociception signalling	578 (symptomatic OA)	64	Caucasian	[180]
	rs8065080	TRPV1	Transient receptor potential cation channel, subfamily V, member 1	Transducer of painful thermal stimuli	7122 (3270 symptomatic knee OA and 3852 controls)	63	Caucasian	[182]
					4950 (1098 asymptomatic knee OA and 3852 controls)	70	Caucasian	
	rs7958311	P2X7	P2X7 purinoceptor	ATP receptor-transducer of pain with neuropathic and inflammatory origin	1329 (743 symptomatic OA and 586 controls)	58	Caucasian and African American	[183]
	rs900414	PCSK6	PACE4 (paired amino acid converting enzyme 4)	Activating pro-aggrecanases	3634 (2068 symptomatic knee OA and 1566 controls)	60	Caucasian	[184]
					2240 (674 asymptomatic knee OA and 1566 controls)	66	Caucasian	
Replication	rs4680	COMT	Catechol-O-methyltransferase	Degradation of catecholamine neurotransmitters such as norepinephrine and dopamine	9556 (3934 symptomatic knee OA and 5622 controls)	62	Caucasian	[179]
					6781 (1159 asymptomatic knee OA and 5622 controls)	65	Caucasian	
	rs6746030	SCN9A	Voltage gated Sodium Channel 1.7 (Nav1.7)	Nociception signalling	1854 (1325 symptomatic OA or TKA and 529 asymptomatic OA)	47	Caucasian	[181]

†Studies reported significant associations between genes and pain;
OA osteoarthritis; TKA total knee arthroplasty.

1.12 Obesity/inflammation in OA pain

Since the end of last century, the relationship between obesity and chronic pain has attracted extensive investigations. There is a sizeable evidence to suggest that obesity and pain adversely impact each other, and obesity is predictive of worse functional and psychological status of chronic pain [185]. Based on a recent US study in one million people, individuals with overweight (BMI, 25–29.9 kg/m^2) reported 20% higher rates of pain, 68% higher for those BMIs of 30–34 kg/m^2, 136% higher for those BMIs of 35–39 kg/m^2 and 254% higher for those BMIs of more than 40 kg/m^2 compared to normal weight group [186]. Evidence from longitudinal studies suggests that obesity is an important risk factor for the development of chronic pain, indicating that obesity is more likely a cause rather than a consequence of pain [187-189].

It has long been assumed that potential mechanism underlying the relationship between obesity and pain may be due to mechanical loading, especially for lower extremities. There is a linear increment of compressive loading across the joint as BMI increases. In knee OA, relative to those with overweight, people classified as class 1 or 2+ obesity have greater peak knee compressive forces [190]. Similarly, weight loss has been shown to be effective in reduction of knee joint forces [191]. Increased joint forces may result in aberrant biomechanical environment; it is, therefore, not surprising to observe greater structural damage in the loading joint in obese individuals, as mentioned above. The evidence of the role of inflammation involved in the link between obesity and pain is accumulating because adipose tissue has been recognised as an endocrine organ responsible for producing and releasing proinflammatory cytokines and adipokines [192]. Research shows an increased level of cytokines and inflammatory markers, such as C-reactive protein (CRP), IL-6, TNF-α and leptin in obese individuals [193, 194]. In addition, the release of inflammatory markers also can be triggered by the breakdown products from structures or tissues damages due to aberrant loading [195, 196]. Elevated levels of these biomarkers lead to enhancing pain severity and its change [197, 198] which in turn stimulate more inflammatory markers release [199]. It has been suggested that inflammation can lead to a lowering of excitation threshold and enhanced responses to suprathreshold stimuli of peripheral nociceptors (peripheral sensitisation) and subsequently developing central nervous system sensitisation with pain hypersensitivity and increased vulnerability to reporting more pain sites [72, 200]. In this context, research should be expanded to investigate the relationship between inflammatory markers and MSP; fat mass could be an ideal surrogate as a source of inflammatory markers in epidemiological studies. **Chapter 7** investigates the relationship between fat mass and MSP. Furthermore, in OA, one constant question for researchers and clinicians is whether pain is an early marker for early OA, albeit with conflicting results of associations between structural damages and single-site knee pain as stated above. It is worth investigating the relationship between MSP and structural damage as MSP may represent a higher level of inflammation and dysfunction in central pain processing, see more details in **Chapter 8**.

Chapter 2: Research questions

Research questions 1, 2 and 3 investigate family history of knee OA and its interaction with body weight on knee structural changes and knee pain.

In a prospective cohort study with offspring having at least one parent with total knee replacement (TKR) for severe primary knee OA and controls with no family history of knee OA examined at baseline, 2.3 and 10.2 years later:

1. What is the relationship between family history of knee OA and knee structural changes over 8-10 years?

    1.1. Do offspring have greater knee structural changes including cartilage defects, meniscal tears, meniscal extrusion and BMLs as compared to controls?

2. Is there a difference in the relationship between body weight and knee cartilage volume loss and defects in offspring and controls?

    2.1. Does body weight in the offspring have a more detrimental effect on knee cartilage than in controls?

3. What is the relationship between family history of knee OA and changes in knee pain over 8 years, as measure by the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) questionnaire?

    3.1. Do offspring have an increased risk of worsening knee pain?

    3.2. Is the relationship between family history of knee OA and changes in knee pain dependent of knee structural factors?

Research questions 4 and 5 investigate obesity on MSP and the predictive value of MSP for the hallmark of knee OA (cartilage volume loss).

In a population-based cohort of aged 50–80 years examined at baseline and 2.6 and 5.1 years later:

4. What are the associations between fat mass, fat mass index and body mass index and MSP over 5.1 years?

    4.1. If there is any association, what is the potential mechanism underlying the association?

5. What is the association between MSP and knee cartilage volume loss over 2.6 years?

    5.1. Do people having more painful sites have greater cartilage volume loss?

    5.2. Which mechanism mediates the association?

Chapter 3: Methodology

3.1 Preclude

This thesis utilised the data from the Offspring study and Tasmanian Older Adult Cohort (TASOAC) study in which a number of outcome factors, exposure factors and covariates have been used. The former was used for Chapter 4, 5 and 6; the latter one Chapter 7 and 8. In these chapters, the study design, study population as well as measurement protocols of studied factors which are common to these two studies are described below. For those studied factors which are unique to each chapter and covariates, the descriptions of those factors are given in details in the corresponding methodology section of the following chapters.

Please be aware that the following chapters are presented in the form in which they were accepted by peer-reviewed journals. Therefore, there might be some differences in the description of methods in accordance with requirements of those journals and requests from journal reviewers. The sample sizes for different chapters vary and are determined by available data for studied factors of the research questions.

3.2 Study population and design

3.2.1 Offspring study

The Offspring Study is a population-based case control study with longitudinal follow-up over ten years. Cases (N=186, mean age 45 years) were enrolled from the eligible adult offspring of subjects who have had TKR performed for primary OA of the knee in Hobart in 1996-2000. Age- and sex-matched controls (N=186) were randomly selected from the electoral roll. People were qualified to be included on the roll if they were: (1) 18 years of age or older, (2) an Australian citizen, (3) an elector entitled to vote at a house of representatives election or qualified to become such an elector. Disqualified from the roll were those who were (1) a member of a state or territory parliament, (2) a citizen or subject of a foreign power, (3) serving a prison sentence of 12 months or more, (4) an undischarged bankrupt or insolvent, (5) holding an office of profit under the Crown (e.g. Public Servant), or (6) a permanent member of the Australian Defence Force. They amounted to about 15% of residents. Participants from either group were also excluded on the basis of contraindication to MRI (including metal sutures, presence of shrapnel, iron filing in eye, and claustrophobia).

The initial measurements were taken from June 2000 to December 2001, a total of 372 participants (186 offspring and 186 controls) aged 26 to 61 years were enrolled. The Phase 2 was conducted 2.3 years (range: 1.8–2.6 years) later, 326 participants (162 offspring and 164 controls) were traced. The Phase 3 was conducted at 10.2 years (range: 9.1–11.4 years), 219 participants (115 offspring and 104 controls) were included.

3.2.2 TASOAC study

The TASOAC is a longitudinal, observational population-based study. The cohort consisted of both men and women and was selected from the electoral roll in Southern Tasmania generated by staff of the Tasmanian Electoral office (total number of people on the roll n=229,593) using sex-stratified simple random sampling without replacement. The eligible cohort consisted of registered electors aged 50–80 years (n=61,715, men/women=29,484/32,231). Institutionalised older adults were excluded because TASOAC was designed to study community-dwelling older adults. Participants were also excluded if they had contraindications for MRI (including pacemakers, implants and claustrophobia), as these tests were required to examine OA progression.

Figure 3-1 shows an overview of subject recruitment and withdrawal during the study period. A total of 2,530 subjects were selected from the roll using 5-year age band information with equal number of men and women. Among them, 395 were deemed unable to participate due to illness or other reasons, and the remainders were contacted via mail by asking whether they would like to participate in the study. Of 2,135 subjects, 1,100 were enrolled in the study and 1,099 attended the first clinic between March 2002 and September 2004 (response rate 57%) at the Menzies Institute for Medical Research, Australia. Follow-up data was collected for 875 eligible participants (80%) at Phase 2 approximately 2.6 years later (range: 1.4–4.8 years) and 769 eligible participants (70%) at Phase 3 approximately 5.1 years later (range: 3.6–6.9 years), respectively. The MRI machine was decommissioned halfway through the follow-up period; therefore, MRI scans were only available for approximately half of the follow-up participants at Phase 2 (n=425 of 875). MRI was not used for the measurement of structures at Phase 3.

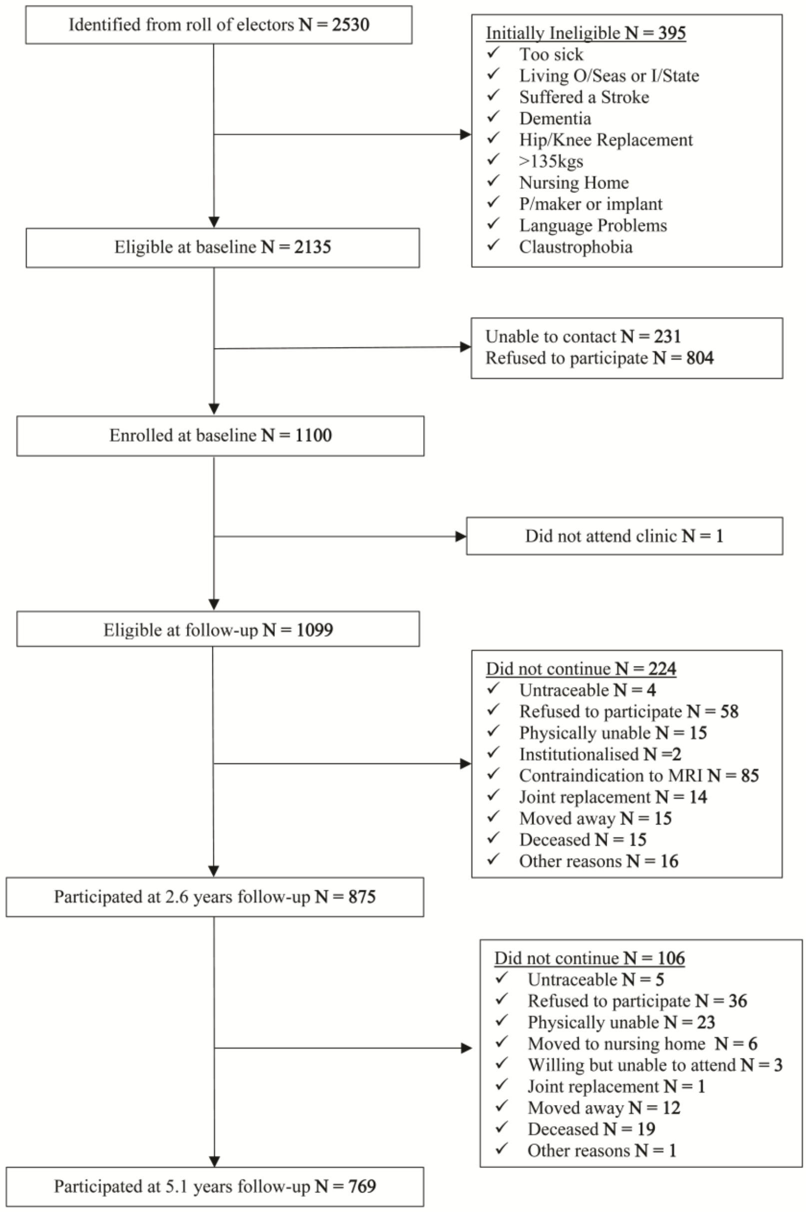

Figure 3-1 Flow chart describing recruitment and withdrawal reasons for TASOAC participants.

3.2.3 Ethics

The Southern Tasmanian Health and Medical Human Research Ethics Committee approved the Offspring and TASOAC study. And all participants provided written informed consent.

3.3 Anthropometrics

Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707) calibrated using a known weight at the beginning of each clinic. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI (kg/m^2) was calculated.

3.4 Magnetic Resonance Imaging

MRI scans of the right knee in the Offspring and TASOAC study were acquired in the sagittal plane on a 1.5–T whole body magnetic resonance unit (Picker, Cleveland, OH) with use of a commercial transmit-receive extremity coil. The following image sequence was used: (1) a T1–weighted fat saturation three-dimensional gradient-recalled acquisition in the steady state; flip angle 30˚; repetition time 31ms; echo time 6.71 ms; field of view 16 cm; 60 partitions; 512×512–pixel matrix, slice thickness of 1.5 mm without an interslice gap; (2) a T2-weighted fat saturation two-dimensional fast spin echo; flip angle 90°; repetition time 3,067 ms; echo time 112 ms; field of view 16 cm; 15 partitions; 228×256–pixel matrix, slice thickness of 4 mm with an inter-slice gap of 0.5–1.0 mm.

3.4.1 Knee cartilage volume

Knee cartilage volume was determined by means of image processing on an independent work station using Osiris (University of Geneva) and measured by two trained and blinded observers as previously described [201]. The volumes of individual cartilage plates (medial tibia and lateral tibia) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312 $\times$ 312 mm and 1.5 mm thickness, continuous sections) for the final three-dimensional rendering. The coefficient of variation (CV) for baseline and follow-up cartilage volume measures was 2.1% for medial tibial, and 2.2% for lateral tibial cartilage [201]. Knee femoral cartilage volume was determined by means of image processing on an independent workstation using Cartiscope ™ (ArthroVision Inc., Montreal, QC, Canada), as previously described [202]. The segmentation of the cartilage-synovial interfaces was carried out with the semi-automatic method under reader supervision and with corrections when needed. Cartilage volume was evaluated directly from a standardized view of three-dimensional cartilage geometry as the sum of elementary volumes. The CV was approximately 1.6% for medial femoral and 2.9% for lateral femoral cartilage at baseline and follow-up [202]. The cartilage volume assessment was done for the medial and lateral condyles delineated by the Blumensaat’s line.

3.4.2 Cartilage defects

Cartilage defects were assessed on T1-weighted MR images at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as previously described [203], as follows: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and base; grade 2 = irregularities on the surface or base and loss of thickness < 50%; grade 3 = deep ulceration with loss of thickness > 50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. A cartilage defect also had to be present on at least two consecutive slices. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. The highest score was used if more than one defect was present on the same site. One observer scored the cartilage defects. Intraobserver repeatability was assessed in 50 subjects with an interval of at least one week between the two measurements. The intraclass correlation coefficients (ICCs) for the Offspring and TASOAC study were 0.93, 0.92, 0.95, and 0.80 at the medial tibia, medial femur, lateral tibia, and lateral femur, respectively.

3.5 Radiographs

A standing anteroposterior semiflexed view of the right knee with 15° of fixed knee flexion was performed. Radiographs were assessed using the Altman atlas [204]. Each of the following was assessed on a scale of 0–3: medial joint space narrowing (JSN), lateral JSN, medial femoral osteophytes, medial tibial osteophytes, lateral femoral osteophytes, and lateral tibial osteophytes. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas. Intraobserver repeatability was assessed in 40 subjects with an interval of at least one week between the two measurements. ICCs ranged from 0.65–0.85. The presence of radiographic osteoarthritis (ROA) was defined as any score ≥1 for JSN or osteophytes.

3.6 Pain measurement

3.6.1 Knee pain

The WOMAC was utilized to assess knee pain. The subscales of the WOMAC which consists of five items (walking on flat surface, going up/down stairs, at night in the bed, sitting/lying and standing upright) with a 10-point scale ranging from 0 (no pain) to 9 (most severe pain) were used for this study [205]. Each item was summed to produce a total pain (0-45) score with higher scores indicating greater pain. A total of score of 1 or greater was considered as presence of knee pain.

3.6.2 Multi-site pain

The location of sites at which the participants experienced pain was measured by self-reported questionnaire. Participants were asked whether they had pain (yes/no) in the following sites at present: neck, back, hands, shoulders, hips, knees or feet. The number of painful sites was summed to create a total number of painful site with a range from 0 to 7, which was then categorised into four groups (non-painful site, 1-2, 3-4, 5-7 painful sites) according to the number of painful site groups with approximately equal numbers of participants reporting one or more painful sites [206]. Number of painful site types was also assessed on a regional basis, with total count of painful upper limb sites created by summing the number of painful upper limb sites (neck, hands and shoulders, range: 0–3), and count of painful lower limb sites created by summing number of hip, knees and feet (range: 0–3).

3.7 Summary of outcome factors, study factors, and covariates

Table 3-1 summarises the variables used in each chapter of this thesis.

Table 3-1 Summary of outcome factors, study factors, and covariates used in this thesis

Chapter	Outcome factors	Study factors	Covariates
4	Cartilage defects BMLs* Meniscal extrusion* Meniscal tear*	Family history of knee OA	Age, sex, BMI, knee injury*, smoking history*, ROA, baseline corresponding structure, and knee structures relevant to each other.
5	Cartilage volume Cartilage defects	Weight Family history of knee OA	Age, sex, height, knee injury*, smoking history*, ROA, bone size* and cartilage defects†.
6	Knee pain	Family history of knee OA	Age, sex, BMI, knee injury*, smoking history*, ROA, cartilage defects, BMLs*, meniscal pathology* and effusion*.
7	MSP	Fat mass* Fat mass index* BMI	Age, sex, height‡, smoking history*, physical activity*, emotional problems*, education level* and employment*.
8	Cartilage volume	MSP	Age, sex, BMI, physical activity*, pain medication*, baseline cartilage volume, cartilage defects and BMLs*.

BMLs bone marrow lesions; OA osteoarthritis; BMI body mass index; ROA radiographic osteoarthritis; MSP multi-site pain
*Measurement protocol described in “Materials and Methods” section of relevant chapter.
†Cartilage defects were further adjusted in the analyses of cartilage volume.
‡Height was only adjusted for fat mass.

3.8 Statistical analysis

T-tests and Chi-square were used to compare differences in means and percentages where appropriate. P values less than 0.05 (two-tailed) were regarded as statistically significant throughout the thesis. The following chapters present the detailed descriptions of statistical analyses. Stata V.12.1 for windows (StataCorp, College Station, Texas, US) and SPSS Statistics (version 20, Chicago IL) were used to perform statistical analyses.

Chapter 4: Familial effects on structural changes relevant to knee osteoarthritis: a prospective cohort study

4.1 Introduction

OA is the most common form of skeletal disorder worldwide and one of the leading causes of pain and disability, resulting in a large social and economic burden [14]. The knee joint is the major site of OA with a prevalence of 30% in those aged 65 and above [207].

It is well-established that knee OA is a multifactorial and highly heterogeneous disease as a result of a complex interaction between local biomechanical factors, such as obesity, mechanical stress and muscle weakness, and systemic factors, such as age, sex and genetics [208]. Genetic factors have been extensively investigated in sibling studies, familial aggregation and twin pair studies, with heritability estimates of approximately 39-65% [127, 131, 209]. GWAS have identified multiple loci involved in the risk of knee OA, but there has been little independent replication [123, 134]; moreover, little is known about the contribution of genetic factors to progression of knee OA over time [41].

Previous studies have shown genetic contributions to knee structures and their changes, including bone size, cartilage volume, cartilage defects and muscle strength [210-213]. There are limited studies on BMLs [214], meniscal extrusion [215] and meniscal tears [216]. These studies have mainly been cross-sectional or short-term with no long-term studies. Therefore, the aim of this study was to describe whether offspring of people having at least one parent with TKR for severe knee OA had a higher rate of change in knee structures of relevance to osteoarthritis in comparison with controls with no knee OA family history over 8 to 10 years.

4.2 Materials and methods

4.2.1 Participants

This study was carried out in southern Tasmania in the capital city of Hobart. The initial measurements were taken from June 2000 to December 2001, and follow-up evaluations were conducted 2 years and 10 years later. Participants were selected from two sources, as described previously [207, 217]. Half of the participants were the adult children (offspring) of participants who had had a TKR performed for primary knee OA at any Hobart hospital from 1996-2000. This diagnosis was confirmed by reference to the medical records of the orthopaedic surgeon and the original radiograph where possible. The other half were controls selected at random from the state Electoral Roll (2000), without a history of knee OA in either parent which was confirmed by history and medical records (TJR). A total of 746 age- and sex-matched controls were identified. Among them, 125 participants who failed to meet the inclusion criteria were excluded, 216 refused to participate this study, 213 were non-contactable or no response, and 192 met the inclusion criteria. Finally, 186 individuals attended all clinical and questionnaires measurements at baseline. Participants from either group were excluded on the basis of contraindication to MRI (including metal sutures, presence of shrapnel, iron filing in eye, and claustrophobia). This study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and all participants provided informed written consent.

4.2.2 Anthropometrics

Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707) calibrated using a known weight at the beginning of each clinic. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI (kg/m^2) was calculated.

4.2.3 Knee injury

Knee injury was assessed at baseline by asking 'Have you had a previous knee injury requiring non-weight-bearing treatment for more than 24 hours or surgery?'.

4.2.4 Radiographs

A standing anteroposterior semiflexed view of the right knee was performed in all participants and scored individually using the Altman atlas for osteophytes and JSN on a scale of 0-3 as previously described [218]. The presence of radiographic OA (ROA) was defined as any score ≥ 1 for JSN or osteophytes.

4.2.5 Knee MRI

An MRI scan of the right knee was performed with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH, US) using a commercial transmit-receive extremity coil. The following image sequences were used: (1) a T1-weighted fat suppression three-dimension gradient-recalled acquisition in the steady state, flip angle 55˚, repetition time 58 ms, echo time 12 ms, field of view 16 cm, 60 partitions, 512 × 512–pixel matrix, slice thickness of 1.5 mm without an interslice gap; (2) a T2-weighted fat saturation two-dimensional fast spin echo, flip angle 90°, repetition time 3,067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228×256–pixel matrix, slice thickness of 4 mm with an inter-slice gap of 0.5–1.0 mm.

4.2.5.1 Cartilage defects

Cartilage defects at baseline and 10 years were assessed as previously described [219] on T1-weighted MR images at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as follows: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and base; grade 2 = irregularities on the surface or base and loss of thickness < 50%; grade 3 = deep ulceration with loss of thickness > 50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. The presence of any cartilage defect was defined as a score of ≥ 2 at any site. The average scores of cartilage defects at the medial tibiofemoral (0 to 8) and lateral tibiofemoral (0 to 8) compartments were used in the study. A cartilage defect score increase was defined as an increase of one or greater at any site.

4.2.5.2 Meniscal extrusion

The extent of meniscal extrusion on the medial or lateral edges of the tibial femoral joint space, not including the osteophytes, was evaluated at baseline and at 10 years for the anterior, body, and posterior horns of the menisci, as previously described [50, 215]. A score from 0 to 2 was used (0 = no extrusion, 1 = partial meniscal extrusion, and 2 = complete meniscal extrusion with no contact with the joint space). The presence of any meniscal extrusion was defined as any score ≥ 1. The scores of anterior, body and posterior horns of medial or lateral menisci were summed to create a total meniscal extrusion score for each of the medial and lateral tibiofemoral compartments which had a possible range from 0-6. A meniscal extrusion score increase was defined as an increase of one or greater at any site.

4.2.5.3 Meniscal tears

At baseline there were only T1-weighted MRI scans which were not suitable for comparison of meniscal tears and BMLs over time. Meniscal tears were assessed at 2 years and 10 years for the anterior, body, and posterior horns of each of the medial and lateral menisci on 0-2 score (0 = no tear, 1 = simple tears of different types: longitudinal, oblique, radial or horizontal signifying loss < 50% area of meniscal tissue, and 2 = macerated tear signifying loss > 50% area of meniscal tissue), as previously described [50, 220]. The presence of any meniscal tear was defined as any score ≥ 1. The scores of anterior, body and posterior horns of medial or lateral menisci were summed to create a total meniscal tear score at the medial/lateral tibiofemoral compartment which had a possible range from 0-6. A meniscal tear score increase was defined as an increase of one or greater at any site.

4.2.5.4 BMLs

BMLs were assessed at 2 years and 10 years on T2-weighted MRI and defined as areas of increased signal adjacent to the subcortical bone at the medial tibial, medial femoral, lateral tibial and lateral femoral sites, as previously described [45]. The readers for BMLs were trained by a radiologist including the differentiation of OA-related BML from similar signal such as contusion/necrosis/edema etc. [221] and consulted the radiologist if there were any doubts. The maximum area (cm^2) of the lesion of different sites was measured, and the BML with the largest size was recorded if more than one lesion was present at the same site. The presence of any BML was defined as any score > 0. The scores of BML at the medial tibiofemoral and lateral tibiofemoral compartments were the sum of the corresponding sites. To adjust for measurement error, a least significant criterion (LSC) [222] was used to define a significant change in BML size (based on previous studies [45, 223]). An increase in BML size was defined as any change greater than the LSC at any site.

4.2.6 Data analysis

T-tests and Chi-square tests were used to compare differences in means and percentage where appropriate. Logistic regression modelling was used to assess the potential relationships between the status of participants (offspring or controls) and four outcomes of knee structural change (increase in cartilage defect score, increase in meniscal extrusion score, increase in meniscal tear score, and increase in BML score), before and after adjustment for common confounders (Step1: age, sex, BMI, ROA, baseline variable of the outcome of interest, history of knee injury and smoking). To exclude a potential effect of other knee structural changes, further adjustment for knee structural changes of relevance to each other (Step 2) in this cohort was also conducted. P values less than 0.05 (2-tailed) or 95% confidence intervals (CIs) not including the “1” point were regarded as statistically significant difference. All statistical analyses were performed using SPSS Statistics (version 20, Chicago IL).

The Hochberg method was used to adjust for multiple testing on regression results [224].

4.3 Results

4.3.1 Participants

A total of 372 participants (186 offspring and 186 controls) aged from 26-61years (mean age of 45 years) were enrolled at baseline. After 2 years (range 1.8-2.6 years), 326 participants (162 offspring and 164 controls) took part and 219 participants (115 offspring and 104 controls) were studied at 10 years (range 9.1-11.4 years). Comparison of the participants lost to follow-up with those included in the present study showed that the proportion of smokers in those lost to follow-up was higher (59% vs. 42%) but no significant differences in other study factors including structural abnormalities was observed (data not shown).

Table 4-1 presents the characteristics of participants who completed 10 years of follow-up. There were no significant differences in terms of age, sex, height, history of knee injury and ROA at baseline between offspring and controls; however, offspring were heavier and had a higher proportion of smokers at baseline.

Table 4-1 Characteristics of participants*

Parameter	Offspring (n=115)	Controls (n=104)	P value
Age, years	44.8 (6.8)	45.8 (6.5)	0.261
Female (%)	60	55	0.435
Height, cm	170.0 (8.5)	168.7 (8.9)	0.270
Weight, kg	80.9 (17.2)	75.1 (14.6)	**0.008**
BMI, kg/m^2	27.9 (5.3)	26.3 (4.5)	**0.018**
Previous knee injury (%)	15	23	0.116
Ever smoking (%)	50	33	**0.008**
Radiographic OA (%)	17	18	0.865
Total cartilage defect score	4.2 (1.2)	3.9 (1.3)	0.095
Any cartilage defect (%)[a]	41	32	0.197
Total meniscal extrusion score	0.2 (0.6)	0.1 (0.3)	0.096
Any meniscal extrusion (%)[b]	10	7	0.423
Total meniscal tear score	0.5 (1.3)	0.3 (0.8)	0.313
Any meniscal tear (%)[b]	21	23	0.744
Total BML score, cm^2	0.3 (0.7)	0.2 (0.5)	0.119
Any BML (%)[c]	55	51	0.529

Bold denotes statistically significant result; BMI, body mass index; OA, osteoarthritis; BML bone marrow lesion;

*Mean (SD) except for percentages; P values determined by t-test or Pearson Chi-square test (where appropriate);

[a]Defined as score ≥ 2 in any compartment;

[b]Defined as score ≥ 1 in any compartment;

[c]Defined as score > 0 in any compartment.

4.3.2 Offspring-control status and knee structural changes

4.3.2.1 Cartilage defects

In this sample, no significant differences were found between offspring and controls in the prevalence of knee cartilage defect as well as the mean tibiofemoral cartilage defect score at baseline (Table 4-1). However, there was a significant difference in the change in cartilage defect score in the medial but not the lateral tibiofemoral compartment (Table 4-2).

In multivariable analysis after adjustment for common confounders, the odds ratio (OR) for medial tibiofemoral cartilage defect increase in the offspring group was 2.5-fold higher than in controls (Table 4-3). This association persisted after further adjustment for other structural factors.

Table 4-2 Differences in knee structural changes between offspring and controls*

Parameter	Offspring (n=115)	Controls (n=104)	P value
Change in cartilage defects†			
Medial compartment	**1.0 (1.5)**	**0.5 (1.2)**	**0.007**
Lateral compartment	0.5 (1.1)	0.5 (1.0)	0.848
Change in meniscal extrusion†			
Medial compartment	**0.3 (0.8)**	**0.1 (0.4)**	**0.027**
Lateral compartment	0.0 (0.0)	0.1 (0.4)	0.181
Change in meniscal tear §			
Medial compartment	**0.4 (1.0)**	**0.1 (0.5)**	**0.012**
Lateral compartment	0.1 (0.7)	0.1 (0.4)	0.778
Change in BML §, cm^2			
Medial compartment	0.2 (0.7)	0.1 (0.5)	0.298
Lateral compartment	0.3 (0.7)	0.1 (0.4)	0.113

Bold denotes statistically significant result; BML bone marrow lesion;

*Change in mean score (SD);

†Change in the knee structure over 10 years;

§Change in the knee structure over 8 years.

Table 4-3 Association between offspring-controls status and any increase in structural abnormality

Outcome	Multivariable analysis‡		Multivariable analysis‡‡	
	OR (95% CI)	P value	OR (95% CI)	P value
Cartilage defects†				
Medial compartment	2.47 (1.28, 4.77)*	0.007	3.04 (1.32, 7.02)*	0.009
Lateral compartment	1.17 (0.59, 2.30)	0.659	1.20 (0.52, 2.75)	0.672
Meniscal extrusion†				
Medial compartment	3.12 (1.07, 9.11)	0.038	10.11 (1.91, 53.48)*	0.007
Lateral compartment	NA	NA	NA	NA
Meniscal tear §				
Medial compartment	3.81 (1.24, 11.73)*	0.020	5.34 (1.42, 20.15)*	0.013
Lateral compartment	NA	NA	NA	NA
BMLs §				
Medial compartment	1.56 (0.77, 3.16)	0.218	0.94 (0.40, 2.22)	0.890
Lateral compartment	1.49 (0.77, 2.90)	0.239	1.33 (0.61, 2.92)	0.476

Bold denotes statistically significant result; OR odd ratio; CI confidence interval; NA not applicable; BMLs bone marrow lesions;

‡Adjusted for age, sex, body mass index, knee injury, smoking history, radiographic osteoarthritis, baseline corresponding structure;

‡‡Further adjusted for all other structural changes in table.

†Change in the knee structure over 10 years;

§Change in the knee structure over 8 years.

*Denotes significant association that passes Hochberg adjustment for multiple testing.

4.3.2.2 Meniscal extrusion

There were no differences in the meniscal extrusion score between the two groups at baseline (Table 4-1), but offspring had a greater increase in average score in the medial but not lateral tibiofemoral compartment (Table 4-2).

In multivariable analysis, offspring had a 3.1-fold higher risk of meniscal extrusion score increase in the medial tibiofemoral compartment and this increased after adjustment for other structures (Table 4-3). This analysis cannot be performed for lateral meniscal extrusion score increase due to the small numbers of cases.

4.3.2.3 Meniscal tear

At 2 years, meniscal tears did not differ between offspring and controls (Table 4-1). Offspring had a greater increase in the score in the medial tibiofemoral compartment over 8 years (Table 4-2).

In multivariable analysis OR for offspring having a meniscal tear score increase as controls was 3.8-fold higher for the medial tibiofemoral compartment. Consistent results were observed after further adjustment for changes in other structures (Table 4-3). Meaningful ORs cannot be obtained for the lateral tibiofemoral compartment as only two participants had increased scores.

4.3.2.4 BML

There were no differences between offspring and controls in terms of the prevalence of BML, BML score at the 2-year visit, and change in BML size in any compartment over 8 years (Table 4-1 and Table 4-2).

After adjustment for multiple testing using the Hochberg method (Table 4-3), the significant associations remained apart from the meniscal extrusion before adjustment for all other structural changes suggesting these results are not due to chance.

4.4 Discussion

To the best of our knowledge, this is the first longitudinal study with a long-term follow-up to examine the relationship between family history of knee OA and knee structural change. We found offspring who had at least one parent with TKR for severe knee OA had an increased risk of worsening of knee structural abnormalities including cartilage defects, meniscal extrusion and tears but not BMLs, suggesting familial factors may be involved in specific knee structural damage. These associations were specific for the medial but not the lateral tibiofemoral compartment, most likely reflecting a predisposition to medial knee OA in their parents. Furthermore, these findings may suggest a greater environmental effect on change in BMLs and change in lateral cartilage defect, and thus they could be modifiable.

Based on familial aggregation and twin studies, there is substantial evidence that genetic factors have an important role in the aetiology of knee OA [127, 131, 209]. Most studies are cross-sectional or case-control studies. There are few studies describing the role of genetic factors in structural knee OA progression. Botha-Scheepers *et al*. [225] reported siblings of proband having progression had 4.3-fold greater risk of radiologic progression of JSN in the knee over 2-year follow-up. Zhai *et al*. [226] also found a strong genetic influence on the progression of ROA in a longitudinal twin study. To date, limited data are available on the roles of genetic factors in the pathogenesis of progression in knee structures prior to end-stage disease [211, 213, 219]. It is known that cartilage defects, BMLs and meniscal pathology play an important role in knee OA, being associated with the adverse structural outcomes [26, 45, 49, 227] and knee pain [228]. Previously, in a larger sample from this cohort, we reported that offspring with family history of knee OA had a higher prevalence of cartilage defects [212] as compared to controls but no differences in the prevalence of meniscal extrusion [215] and tears [216]. However, in the subsample of participants with available longitudinal data, there was no statistical difference in cartilage defects at baseline most likely due to the smaller sample size. Nonetheless, despite the smaller sample size available for long-term follow-up, we showed that offspring had a greater increase in multiple structural abnormalities, independent of baseline structures. The only exception was no effect for BMLs but the ORs were around one, suggesting that this is not a power issue.

Offspring had an elevated risk for an increase in cartilage defects, meniscal extrusion and tears over 8 to 10 years in the medial tibiofemoral compartment after adjustment for age, sex, BMI, ROA, knee injury, smoking and other knee structural factors of relevance to each other, suggesting familial effects on progression in these structures and the influence of familial factors is compartment-specific. These results are comparable with an earlier familial study that reported a 3.2-fold increased risk of prevalence of ROA in siblings [229], but appear to be greater than those reported by Neame *et al*. [230]. The difference in results may be explained by the difference in study population of the study by Neame *et al*. where siblings were compared with the subjects from knee pain studies. This could overestimate the prevalence of knee OA among this group, and therefore resulting in a lower risk of prevalence of knee OA in siblings than ours. Additionally, a previous study with 2-years follow-up in this population found that offspring had a greater increase in the medial cartilage defects [219]. However, it is unknown which genes underlie the progression of cartilage defects.

Although meniscal pathology may be the result of knee injury, knee malalignment and high BMI [227], there are data supporting a genetic contribution to meniscal pathology [216, 231, 232]. Sun *et al*. [231] reported significantly higher levels of gene expression responsible for biological processes in OA meniscal cells as compared to normal meniscal cells, which suggests that aberrant expression of genes may be involved in meniscal pathology. Furthermore, in a longitudinal study by Englund *et al*., people with bony enlargement of finger joints had an increased risk of the development of meniscal pathology, implying that meniscal pathology may be affected by genetic factors, given a strong genetic component of bony enlargement of finger joints [232]. A previous cross-sectional study from our group found that offspring with a family history of knee OA had a two-fold higher risk for the presence of lateral anterior and posterior meniscal tear as compared to controls, indicated genetic factors may be a risk factor for meniscal tears [216]. The present study supports independent familial effects on each knee structural change as the higher risk of progression in cartilage defects, meniscal extrusion and tears in offspring was observed after adjustment for known confounders and for each other. All of these factors have been considered part of the MRI diagnosis of knee OA [233] and thus could be identified early in ‘at risk’ people.

The present study failed to detect any differences in the progression of BMLs in any compartment between offspring and controls, which contrasts with a previous sib pair study in this sample that showed a heritability estimate of 50% for prevalent BMLs [214].Thus, it may be that familial factors are only responsible for the initial development of BMLs but not for progression.

Despite some strengths of this study, including its longitudinal design, long-term follow-up, and the use of MRI to evaluate knee structural changes, there are several potential limitations. Firstly, although some potential confounders have been adjusted in the current study, we cannot rule out the possibility that unidentified confounders or unmeasured factors influence the risk for change in knee structures independently of family history of knee OA. For instance, although results of the association between smoking and knee OA have been inconsistent from previous studies and a recent meta-analysis including 38 studies also failed to demonstrate a causal relationship between them [234], there was a high proportion of smokers in those lost to follow-up and offspring at baseline, this may suggest more psychosocial problems in those people. Furthermore, although knee alignment was not assessed in the present study, a previous study [235] from this cohort demonstrated that this factor is not related to knee structural change, suggesting the absence of alignment assessment is not a limitation for this study. Secondly, because of the rarity of lateral meniscal tears and extrusion, we were unable to determine if familial factors have effects on progression in lateral compartment. Thirdly, genetic factors may play different roles across different ethnic groups [236]. The present study only recruited Caucasians, thus it is inappropriate to extrapolate to other ethnic groups. Lastly, the loss to follow-up may lead to bias; however, there were no significant differences between the participants included in this study and the rest of the cohort in many studied factors apart from only a slightly higher percentage of smokers in those lost to follow-up and adjusting for this factor did not influence results.

In conclusion, offspring with family history of knee OA have an increased risk of progression of multiple knee structures in the medial tibiofemoral compartment compared to controls suggesting pleiotropic familial effects.

Chapter 5: The interaction between weight and family history of total knee replacement with knee cartilage: a 10-year prospective study

5.1 Introduction

Knee OA is the most common form of arthritis worldwide and affects an estimated 30% of individuals aged over 65 years, often leading to pain, disability and reduced quality of life [15, 237]. Despite this, its etiology remains poorly understood.

Knee OA is considered to be a multifactorial and heterogeneous disease affected by genetic and multiple environmental factors [123, 238]. Previous family-based studies have shown a strong genetic basis for OA, with the estimated heritability of approximately 39-65% [239, 240]. There are 11 loci associated with OA now identified in GWAS, although the effect sizes are small [15]. Being overweight or obese is one of the strongest environmental risk factors for the development and progression of radiographic knee OA [2, 148]. A recent meta-analysis has shown an over two-fold increased risk of radiographic knee OA in those who are overweight or obese [148]. An earlier study has reported that the effect of variation in the FTO gene on increased risk of OA is mediated solely through its effect on BMI [241].

Cartilage volume and defects, measured by MRI, are examples of the early structural changes of pre-radiographic knee OA. Both predict clinically relevant endpoints such as knee replacement [27, 28, 242]. Most studies investigating the effect of weight/BMI on knee cartilage have mainly been cross-sectional or short-term follow-up studies and have reported generally consistent detrimental relationships of increasing weight with cartilage defects but not cartilage volume [150]. Given that overweight/obesity is a strong risk factor for knee OA, and genetic factors are implicated in the pathogenesis of knee OA, it is important to understand how genetic factors interact with weight to influence the risk of pre-radiographic knee OA. The aim of this longitudinal study was, therefore, to examine the effects of weight on knee cartilage volume and defects over 10 years in offspring having at least one parent with a TKR for severe primary knee OA, and in controls with no family history of knee OA.

5.2 Methods

5.2.1 Participants

This study was carried out in Hobart, Tasmania. Baseline measurements were taken from June 2000 to December 2001, and follow-up evaluations were conducted after approximately 2 and 10 years. Participants were selected from two sources, as described previously [207, 217]. Half of the participants were the adult children (offspring) of people who had a TKR performed for primary knee OA (defined as OA lacking known cause) at any Hobart hospital from 1996-2000. This diagnosis was confirmed by reference to the medical records of the orthopaedic surgeon and the original radiograph where possible. The other half were controls selected at random from the state Electoral Roll (2000), without a history of knee OA in either parent which was confirmed by history and medical records. Participants from either group were excluded on the basis of contraindication to MRI (including metal sutures, presence of shrapnel, iron filing in eye, and claustrophobia). The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and all participants provided informed written consent.

5.2.2 Anthropometrics

Anthropometrics were measured at baseline, 2 and 10 years. Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707) calibrated at the beginning of each clinic. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI (kg/m^2) was calculated.

5.2.3 History of knee injury and smoking

Knee injury was assessed at baseline by asking ‘Have you had a previous knee injury requiring non-weight-bearing treatment for more than 24 hours or surgery?’. Smoking history was assessed at baseline by asking ‘Have you ever smoked at least seven cigarettes, cigar or pipes every week for at least three months?’.

5.2.4 Radiographs

A standing anteroposterior semiflexed view of the right knee was performed at baseline and scored using the Altman atlas for osteophytes and JSN on a scale of 0-3 as previously described [218]. The presence of ROA was defined as any score ≥ 1 for JSN or osteophytes.

5.2.5 Knee MRI

An MRI scan of the right knee was performed with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH, US) using a commercial transmit-receive extremity coil at baseline, 2-year and 10-year follow-up. The following image sequences were used: a T1-weighted fat suppression three-dimension gradient-recalled acquisition in the steady state, flip angle 55°, repetition time 58 ms, echo time 12 ms, field of view 16 cm, 60 partitions, 512×512–pixel matrix, slice thickness of 1.5 mm without an interslice gap.

5.2.5.1 Cartilage volume

Knee cartilage volume was determined by means of image processing on an independent work station using Osiris (University of Geneva) as previously described [243, 244]. The volumes of individual cartilage plates (medial tibia and lateral tibia) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then resampled by means of bilinear and cubic interpolation (area of 312 $\times$312 mm and 1.5 mm thickness, continuous sections) for the final three-dimensional rendering. The CV for cartilage volume measures was 2.1% for medial tibial, and 2.2% for lateral tibial cartilage [243]. Knee femoral cartilage volume was determined by means of image processing on an independent workstation using Cartiscope ™ (ArthroVision Inc., Montreal, QC, Canada), as previously described [50, 245, 246]. The segmentation of the cartilage-synovial interfaces was carried out with the semi-automatic method under reader supervision and with corrections when needed. Cartilage volume was evaluated directly from a standardized view of three-dimensional cartilage geometry as the sum of elementary volumes. The CV was approximately 2.0% [245]. The cartilage volume assessment was done for the medial and lateral condyles delineated by the Blumensaat's line [246]. The medial and lateral tibiofemoral cartilage volume created for this study were the sum of the cartilage volume of the corresponding sites.

5.2.5.2 Cartilage defects

Cartilage defects were assessed as previously described [219] on T1-weighted MRI at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as follows:

grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and base; grade 2 = irregularities on the surface or base and loss of thickness <50%; grade 3 = deep ulceration with loss of thickness >50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. The ICCs ranged from 0.89–0.90 for intra-observer repeatability. Interobserver reliability was assessed in 50 MR images and yielded an ICC of 0.85–0.90 [247]. The presence of any cartilage defect at medial or lateral tibiofemoral compartment was defined as a score of ≥2 at either tibial or femoral site.

5.2.5.3 Bone area

The tibial plateau bone area were determined, as described previously [248]. Medial and lateral tibial plateau area was determined by creating an isotropic volume from the three input images closest to the joint after reformatting in the axial plane. The areas of the medial and lateral tibial plateaus were then directly measured from these images. The CV was 2.3% and 2.4% for the medial and lateral tibial plateau [248].

5.2.6 Data analysis

The continuous and categorical variables were respectively presented as Mean ± standard deviation (SD) and percentages. T-test and Chi-square tests were used to compare the differences in means and percentages where appropriate. Ordinal $\chi 2$ test (Kruskal-Wallis test) was used to test if there was a trend of prevalence of cartilage defects across category of body mass index. Longitudinal data were analysed using mixed-effects models that take the dependence of repeated observations within participants into account and use all data, thus the participants who were lost to follow-up also were included in the analyses. Linear and poisson mixed-effects model with random intercept for participants were used to assess the potential associations of weight with cartilage volume and defects, respectively, before and after adjustment for age, sex, height, smoking history, knee injury, corresponding bone size and ROA where appropriate. Interaction between weight and offspring-control status was tested using full longitudinal dataset in the model and was found significant ($P \leq 0.2$ is considered as statistical significance [249]), suggesting that the effect of weight on cartilage volume/defects was different in offspring and control groups. We, therefore did subgroup analyses in offspring and control group separately. Furthermore, we also performed separate mixed-effect models with random intercept for participants and 'weight × time' interaction term to determine their temporal associations. Body weight was standardised by dividing by SD; therefore, all beta or relative risk (RR) represented cartilage volume or defects associated with per SD increase of body weight. We also estimated how much the genetic 'load' contributes to the risk of cartilage defects due to overweight/obesity through calculating the R square change in the model before and after adjustment for offspring-control status. Inverse probability weighting was used to determine whether loss to follow-up biased our results. All statistical analyses were performed using Stata V.12.1 for windows (StataCorp, College Station, Texas, US). $P \leq 0.05$ (two-tailed) was regarded as statistically significant.

5.3 Results

The characteristics of participants at each time-point are presented in Table 5-1. A total of 372 participants with 186 offspring and 186 controls aged 26–61 years (mean age of 45 years) participated in this study at baseline. 326 and 219 participants underwent follow-up assessment at 2 and 10 years, respectively. No significant differences between participants followed (n=219) and those lost to follow-up (n=153) in all baseline studied factors apart from a higher proportion of smoking history in loss to follow-up group (59% vs. 42%). There were increases in mean weight/BMI over 10 years. Medial and lateral tibiofemoral cartilage volume decreased and the prevalence of cartilage defects increased from baseline to 10 years. At baseline, there were no significant differences between offspring and controls in demographics, ROA, bone size, cartilage volume, defects (scores and prevalence); however, offspring weighted more and had a higher BMI compared with controls. There was increase in weight over 10 years in both group (Table 5-2).

Table 5-1 Characteristics of participants at each time-point*

Characteristics	Baseline (n=372)	Phase 2 (n=326)	Phase 3 (n=219)
Follow-up, years	0	2.3±0.4	10.2±0.5
Age, years	45.2±6.9	47.5±8.6	55.4±6.6
Female (%)	58	58	58
Height (cm)	169.1±8.5	168.6±8.6	168.6±8.8
Weight (kg)	77.5±15.5	78.7±16.2	80.1±17.0
Body mass index (kg/m^2)	27.1±4.7	27.6±5.0	28.1±5.3
Ever smoked (%)†	49		
Previous knee injury (%)†	20		
Radiographic osteoarthritis (%)†	21		
Medial bone area (cm^2)†	17.4±2.7		
Lateral bone area (cm^2)†	12.0±2.0		
Knee cartilage volume (ml)			
Medial tibiofemoral	6.8±1.7	6.3±1.6	5.5±1.5
Lateral tibiofemoral	7.4±1.9	6.9±1.8	6.3±1.7
Knee cartilage defect prevalence(%)‡			
Medial tibiofemoral	25	29	46
Lateral tibiofemoral	17	22	30

*Values are the Mean±SD except for percentages;

†Variables were measured at baseline.

‡Defined as defect score≥2 in tibial or femoral compartment.

Table 5-2 Characteristics of participants*

Characteristics	Controls (n=184)	Offspring (n=183)	P value
Age, years	45.3±6.9	45.1±6.9	0.849
Female (%)	58	57	0.798
Height (cm)	169.0±8.7	169.1±8.3	0.917
Weight (kg)	**75.6±14.9**	**79.5±15.8**	**0.018**
Body mass index (kg/m^2)	**26.4±4.4**	**27.7±4.9**	**0.007**
Ever smoked (%)	44	53	0.085
Previous knee injury (%)	22	17	0.198
Radiographic osteoarthritis (%)	17	25	0.138
Medial bone area (cm^2)	17.2±2.8	17.7±2.6	0.089
Lateral bone area (cm^2)	11.9±2.1	12.0±2.0	0.608
Knee cartilage volume (ml)			
Medial tibiofemoral	6.7±1.8	6.9±1.5	0.273
Lateral tibiofemoral	7.2±1.9	7.6±1.9	0.106
Knee cartilage defect score (0–8)			
Medial tibiofemoral	2.0±0.8	2.2±0.8	0.129
Lateral tibiofemoral	1.9±0.7	2.0±0.8	0.245
Knee cartilage defect prevalence (%)†			
Medial tibiofemoral	21	29	0.153
Lateral tibiofemoral	17	17	0.907
Change in weight (kg)	2.1±6.0	1.9±8.9	0.865

*Values are the Mean±SD except for percentages;

†Defined as defect score≥2 in tibial or femoral compartment.

Table 5-3 describes the baseline characteristics of participants by median weight in offspring and controls. 367 participants (184 controls and 183 offspring) with complete data were included for the analyses. There were no differences between participants below and above or equal median weight in terms of age, smoking history and prevalence of ROA in either controls or offspring. In both offspring and controls, participants above or equal median weight were more likely to be men, taller, have a higher rate of knee injury and greater bone size and cartilage volume. Cartilage defect score (but not prevalence) was greater in participants above or equal median weight than participants below median weight in offspring; however, these were not different in controls. The prevalence of medial tibiofemoral cartilage defects increased with each category of BMI in offspring at each phase but became less over time (P for trend<0.05 at baseline and Phase 2 and P=0.126 for Phase 3), and overweight/obese offspring had a higher prevalence of medial tibiofemoral cartilage defects than overweight/obese controls (Figure 5-1). Furthermore, compared with those with weight loss, participants with weight stable and weight gain had a higher proportion with an increase in medial tibiofemoral cartilage defects (Figure 5-2).

Table 5-3 Characteristics of participants at baseline*

Characteristics	Controls (n=184)			Offspring (n=183)		
	Weight < median (n=95)	Weight ≥ median (n=89)	P value	Weight < median (n=86)	Weight ≥ median (n=97)	P value
Age, years	44.8±7.0	45.8±6.9	0.300	44.4±7.4	45.7±6.3	0.208
Females (%)	**81**	**34**	**<0.001**	**79**	**37**	**<0.001**
Height (cm)	**164.9±6.8**	**173.5±8.3**	**<0.001**	**165.2±5.7**	**172.6±8.7**	**<0.001**
Ever smoked (%)	41	47	0.402	56	51	0.474
Previous knee injury (%)	17	28	0.067	**10**	**23**	**0.028**
Radiographic knee OA (%)	14	20	0.362	20	30	0.228
Medial bone area (cm^2)	**15.9±2.2**	**18.6±2.7**	**<0.001**	**16.2±1.8**	**19.1±2.5**	**<0.001**
Lateral bone area (cm^2)	**11.0±1.6**	**13.0±2.0**	**<0.001**	**11.1±1.4**	**12.9±2.1**	**<0.001**
Knee cartilage volume (ml)						
Medial tibiofemoral	**6.0±1.4**	**7.5±1.9**	**<0.001**	**6.2±1.2**	**7.6±1.5**	**<0.001**
Lateral tibiofemoral	**6.3±1.5**	**8.1±1.9**	**<0.001**	**6.6±1.3**	**8.5±1.9**	**<0.001**

Knee cartilage defect score (0–8)						
Medial tibiofemoral	1.9±0.9	2.2±0.7	0.056	**2.0±0.7**	**2.4±0.8**	**0.010**
Lateral tibiofemoral	1.8±0.7	1.8±0.7	0.753	**1.8±0.7**	**2.1±0.8**	**0.033**
Knee cartilage defect prevalence (%)†						
Medial tibiofemoral	17	25	0.299	20	37	0.055
Lateral tibiofemoral	17	17	1.000	14	20	0.407

Bold denotes statistically significant results in either offspring or control group determined by t test or Pearson χ2 test (where appropriate). BMI, bone mass index; OA, osteoarthritis;

*Values are the Mean±SD except for percentages;

†Defined as defect score≥2 in tibial or femoral compartment.

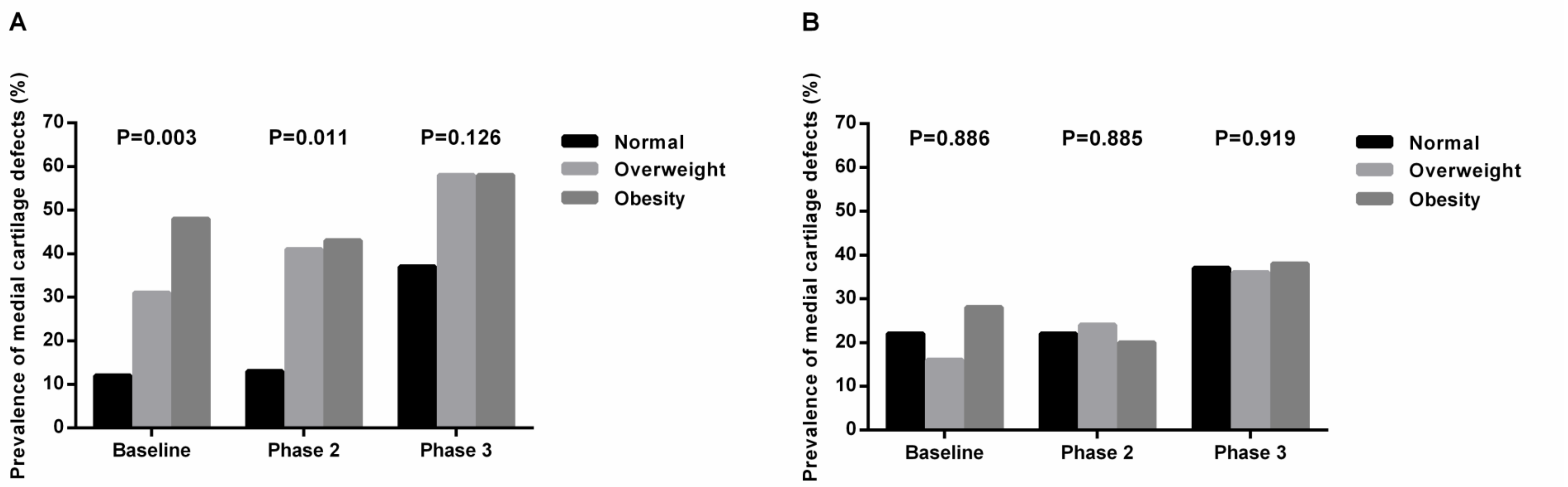

Figure 5-1 Concurrent associations between category of body mass index and prevalence of medial tibiofemoral cartilage defects at each phase in (A) offspring; (B) controls. The bars represent prevalence of medial tibiofemoral cartilage defects in normal, overweight and obese group (Normal: body mass index<25; Overweight: 25≤body mass index<30; Obesity: body mass index≥30). P for trend determined by Kruskal-Wallis test.

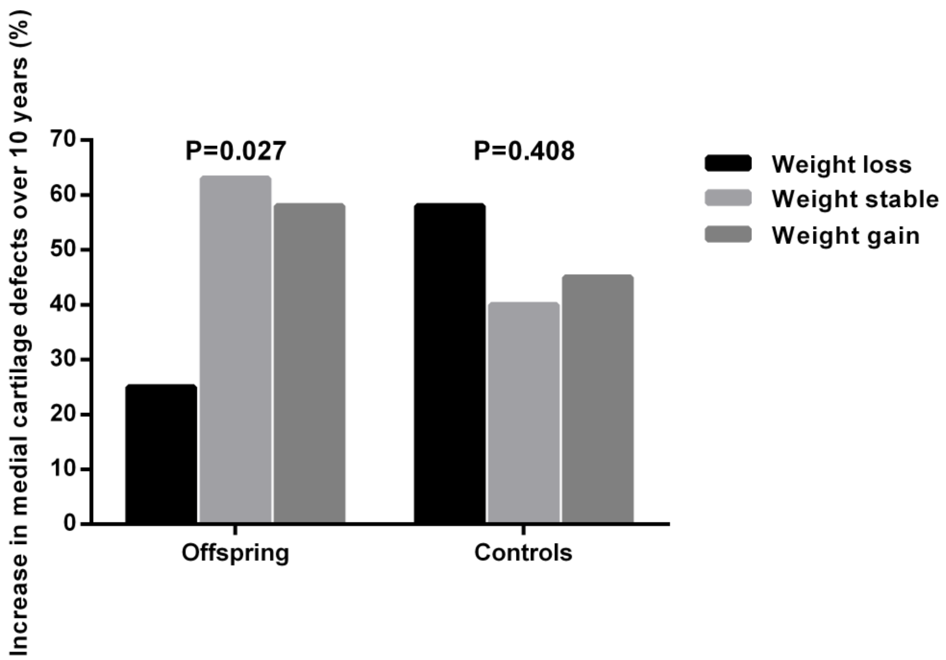

Figure 5-2 Increase in medial tibiofemoral cartilage defects among those with weight loss, weight stable and weight gain over 10 years. An increase in medial tibiofemoral cartilage defect was defined as an increase of 1 or greater. Weight loss, weight stable and weight gain were defined as those who lost relative weight change of 5% or greater, those who gained or lost less 5% and those who gained 5%. P for trend determined by Kruskal-Wallis test.

Table 5-4 shows the association between weight and knee tibiofemoral cartilage volume in offspring and controls over 10 years, based on mixed-effects linear regression. In multivariable analyses with adjustment for age, sex and height; weight is negatively associated with medial but not lateral tibiofemoral cartilage volume which approached but did not reach statistical significance ($P=0.061$) in the offspring. This association persisted after further adjusting for smoking history, previous knee injury, ROA, bone size and cartilage defects. There was a significant weight $\times$ time interaction with lateral tibiofemoral cartilage volume over 10 years (interaction: $\beta=-0.02$ ml, $P<0.001$), as shown in Figure 5-3. This result demonstrates that the association between weight and lateral cartilage volume became more negative as time increased; namely, the decrease in lateral tibiofemoral cartilage volume per SD increase in weight was 0.02 ml greater every year. In contrast, there were no significant associations of weight with cartilage volume loss at any compartments in controls.

Table 5-4 Association between weight and knee tibiofemoral cartilage volume over 10 years

	Increase in weight per SD*			
	Model 1†		Model 2‡	
	β	95% CI	β	95% CI
Offspring				
Medial tibiofemoral	**-0.21**	**-0.38, -0.04**	**-0.28**	**-0.49, -0.07**
Lateral tibiofemoral §	-0.17	-0.35, 0.01	-0.19	-0.38, 0.01
Controls				
Medial tibiofemoral	-0.12	-0.33, 0.09	-0.21	-0.47, 0.04
Lateral tibiofemoral	-0.07	-0.26, 0.12	-0.11	-0.33, 0.10

Bold denotes statistically significant result. β beta coefficient; CI confidence interval;

*β (95%CI): regression coefficient (95% confidence interval) estimated by mixed-effects linear regression representing the change in mean cartilage volume associated with a per SD change in weight;

†Adjusted for age, sex and height;

‡Further adjusted for ever smoking, previous knee injury, radiographic knee osteoarthritis, corresponding bone size and cartilage defects.

§There is an interaction between weight and time.

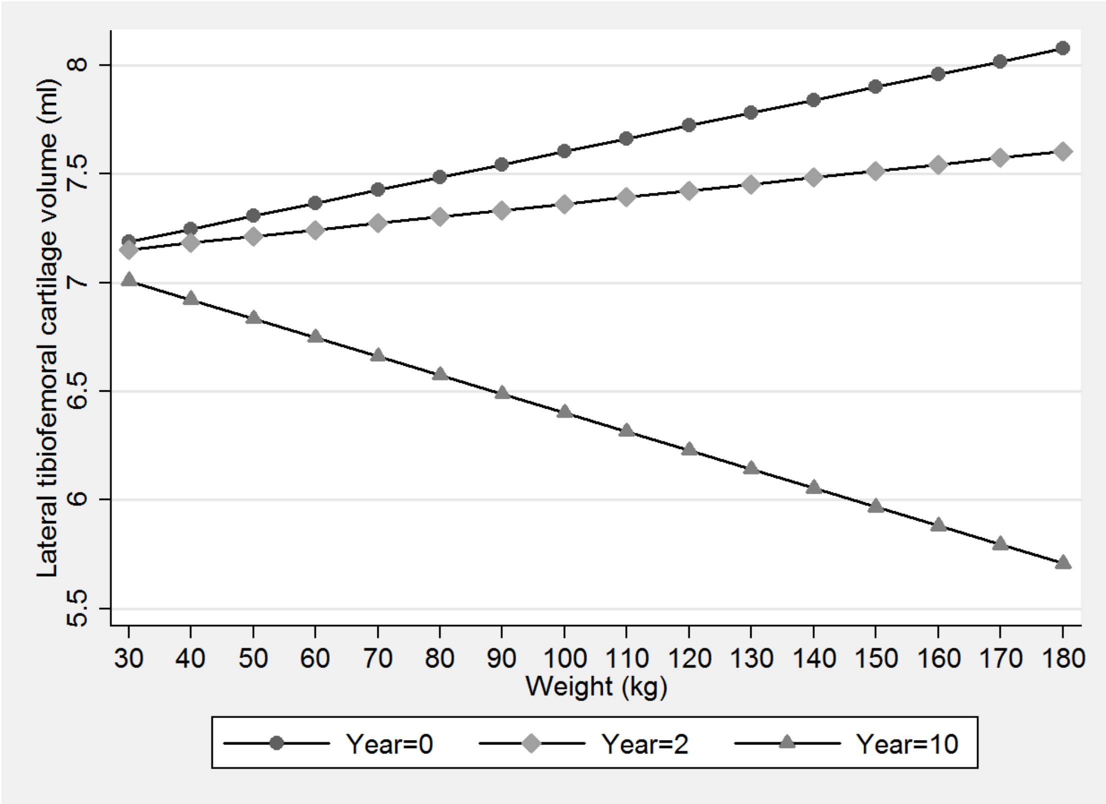

Figure 5-3 Interaction between weight and time on lateral knee cartilage volume in offspring. There was a significant interaction between weight and time on lateral knee cartilage volume. The effect of increasing weight on lateral knee cartilage volume becomes more negative over time. The model adjusted for common covariates including age, sex, height, smoking, knee injury, radiographic knee osteoarthritis, bone size and cartilage defects.

Table 5-5 shows the association between weight and cartilage defects in offspring and controls over time, based on mixed-effects poisson regression. After adjustment for age, sex and height, the odds of medial and lateral cartilage defects increased with increasing weight at all time-points for offspring. These associations remained significant after further adjustment for other potential confounders. Furthermore, there was a significant weight × time interaction for medial cartilage defects, indicating the association between weight and medial cartilage defects decreased over time. Similarly, we did not observe significant associations between weight and cartilage defects in controls. After using inverse probability weighting, our results were very similar.

Genetic 'load' contributing to the risk of medial tibiofemoral cartilage defects due to overweight/obesity was also estimated. Before and after adjustment for offspring-control status, R^2 increased from 0.56 to 0.66 (approximately 18% increase, P=0.011), suggesting that genetic 'load' accounts for 18% of risk of cartilage defects due to overweight/obesity.

Table 5-5 Association between weight and knee tibiofemoral cartilage defects over 10 years*

	Increase in weight per SD			
	Model 1†		Model 2‡	
	RR	95% CI	RR	95% CI
Offspring				
Medial tibiofemoral §	**1.32**	**1.14, 1.54**	**1.27**	**1.07, 1.51**
Lateral tibiofemoral	**1.28**	**1.05, 1.57**	**1.24**	**1.00, 1.54**
Controls				
Medial tibiofemoral	1.06	0.81, 1.39	1.05	0.79, 1.38
Lateral tibiofemoral	0.77	0.52, 1.14	0.74	0.50, 1.11

Bold denotes statistically significant result. RR relative risk; CI confidence interval;

*RR (95%CI): relative risk (95% confidence interval) estimated by mixed-effects poisson regression;

†Adjusted for age, sex and height;

‡Further adjusted for ever smoking, previous knee injury and radiographic knee osteoarthritis;

§There is an interaction between weight and time.

Appendices 5-1 Association between weight and knee tibiofemoral cartilage volume/defects over 10 years in the whole population*

	Increase in weight per SD			
	Model 1†		Model 2‡	
Cartilage volume	β	95% CI	β	95% CI
Medial tibiofemoral	**-0.17**	**-0.30, -0.04**	**-0.28**	**-0.45, -0.12**
Lateral tibiofemoral §	-0.12	-0.25, 0.01	**-0.16**	**-0.30, -0.01**
Cartilage defects	RR	95% CI	RR	95% CI
Medial tibiofemoral §	**1.27**	**1.12, 1.45**	**1.21**	**1.05, 1.39**
Lateral tibiofemoral	1.13	0.94, 1.36	1.05	0.87, 1.29

Bold denotes statistically significant result. RR relative risk; CI confidence interval;

*RR (95%CI): relative risk (95% confidence interval) estimated by mixed-effects poisson regression;

†Adjusted for age, sex and height;

‡Further adjusted for ever smoking, previous knee injury and radiographic knee osteoarthritis. For cartilage volume, cartilage defects were additionally adjusted;

§There is an interaction between weight and time.

5.4 Discussion

This study found that detrimental associations between weight and knee cartilage over 10 years in this mid-life population were consistently stronger in the offspring of people with TKR for severe primary knee OA, as compared to controls with no family history of knee OA. This suggests possible genetics-environment interaction with regard to overweight/obesity in the pathogenesis of early-onset knee OA.

Although overweight/obesity is a well-established risk factor for the development of radiographic knee OA [2, 148], the results from previous studies on knee cartilage on MRI are mixed [150]. The current study found medial cartilage volume to be detrimentally associated with weight in the whole population (Appendices 5-1). Although this contrasts to some previous findings, with our initial reports from this cohort [250] and similar studies [152, 251-257], showing no effects of weight/BMI on cartilage volume either in cross-sectional or longitudinal studies, our results are consistent with some studies. For instance, an earlier cross-sectional study in an older population from our group found a significant negative association of BMI with cartilage volume [159]. A previous study with 1-year follow-up showed that weight loss in obese individuals was associated with reduced medial femoral cartilage thickness loss [258]. Furthermore, Teichtahl *et al.* [259] reported that weight loss was associated with reduced medial tibial cartilage loss in a 2.3-year follow-up study. Similarly, in a 4-year follow-up study by Bucknor *et al.* [260], they found that weight gain was associated with increased progression of cartilage lesions. These inconsistencies may be attributable to the differences in characteristics of population included (sample size, age, and the proportion of ROA), study design, follow-up time.

Interestingly, compared to previous cross-sectional or longitudinal studies on older populations [251-256], those studying younger population found no association between weight/BMI and cartilage volume [152, 250, 257]. This may reflect the effects of aging making knee joints more susceptible to body weight [261]. This is supported by our finding of significant interaction between weight and time on lateral cartilage volume, suggesting the effects of weight on cartilage volume was increasing with age. In agreement with prior studies which found more consistent detrimental relationships for cartilage defects [152, 153, 159, 253, 262], we found that weight was associated with increasing risk of medial cartilage defects. Despite the fact that this association became weaker with greater age, the RR of increasing weight on medial cartilage defects was still greater than one at 10 years. This could be attributable to ceiling effects on cartilage defects with age. Taken together, the current study extends previous observations into a long-term follow-up, and provides a strong support for the detrimental effects of weight gain on knee cartilage.

Knee OA is a complex condition in which both genetic and environmental factors result in its development and progression [163]. There is mounting evidence to support the gene-environment interaction with risk of knee OA, although the mechanism remains unclear. In a previous study with 2-year follow-up from in this population, we found that smoking interacts with family history of knee OA to influence the susceptibility to change in knee cartilage and cartilage defect development [263]. A previous epidemiologic study also showed that the odds of having familial OA is greater in those with a higher BMI [264]. Muthuri *et al.* [163] reported that a significant interaction between overweight and transforming growth factor-β1 (TGF-β1) gene in the risk of knee OA. Panoutsopoulou *et al*. [241] found that the obesity-related gene (FTO) increased the risk of OA and this association is mediated by BMI, indicating that FTO gene has its effects on OA solely through obesity. Another study based on GWAS data which investigated the genetic overlap between BMI and OA, further supports the conclusion that FTO gene is shared in the association between BMI and OA [265]. Estimated genetic 'load' from this study not only further supports genetic factors involved in the effect of BMI on cartilage damage, but indicates a larger effect of environmental component on cartilage damage (82% of the effect on cartilage damage is due to environmental component), and hence cartilage damage may be modifiable. Despite no current evidence of a genetic overlap between weight and cartilage, the current findings that the effect sizes of weight on cartilage volume and defects over 10 years in offspring were significantly and consistently higher than that in controls, together with these previous studies, may suggest gene-environment interaction between weight and family history of knee OA. Though speculative, one possible explanation may be that subtle alterations in extracellular matrix function attributable to genes involved in regulation of extracellular matrix protein in offspring, render offspring more likely to be affected by environmental factors, even small amounts of weight gain [123].

The current study found that increase of weight is statistically and negatively associated with knee cartilage volume at the medial but not the lateral compartment in offspring. These results are in line with previous studies which found that weight loss was associated with reduction in cartilage loss at medial compartment [258, 259], possibly because this is the main weight-bearing site in the knee. We did observe a tendency towards an adverse effect of weight gain on knee cartilage volume at the lateral compartment which approached but did not reach statistical significance, and the effect of increase of weight on lateral cartilage volume loss increases with time. It may be that a significant association at lateral compartment may be detectable over a longer time-frame.

There are several potential limitations of this study. First, we adjusted for available potential confounders but we cannot rule out the influence of other knee structures such as BMLs and meniscal tears as T2-weighted images were not available at baseline. However, while adjusting for meniscal extrusion which is closely correlated with meniscal tears and has similar effects with meniscal tears on increased risk for cartilage volume loss as well as BMLs at phase 2 and 3 [232], our results remained largely unchanged; so it is unlikely that adjusting for these factors would change our results. Second**,** loss to follow-up may result in bias; however, there were no significant differences between those who completed the follow-up and the rest of the cohort in terms of studies factors except for a slightly higher percentage of smokers in those lost to follow-up. Our results did not change after using inverse probability weighting. Also, mixed-effect models were used for analyses which consider the dependence of repeated observations within participants and include the data of participants who were lost to follow-up. Third, our results may be influenced by worsening disease or co-morbidities related to weight gain over such a long period. Individuals with worsening disease or co-morbidities are more likely to be loss to the follow-up; this may underestimate our results. However, we did not screen co-morbidities, and thus were unable to assess their influence on our results.

In conclusion, the adverse effects of increasing weight are stronger in the offspring of people with knee replacement for knee osteoarthritis than that in controls without a family history of knee OA suggesting genetics-environment interaction with regard to overweight/obesity in the pathogenesis of knee osteoarthritis particularly in the early stages.

Chapter 6: The offspring of people with a total knee replacement for severe primary knee osteoarthritis have a higher risk of worsening knee pain over 8 years

6.1 Introduction

OA is the most common form of arthritis in western countries [14]. The knee joint is the major site of OA with a prevalence of 30% in those aged 65 and above [207]. Pain in the knee is the most common presenting symptom of knee OA, but it also occurs due to other musculoskeletal diseases [266, 267]. Knee pain leads to significant restrictions in function that prevent OA patients from engaging in their daily activities [268], and may eventually require surgical treatment [269]. It is reported that approximately 21-35% of people aged 45 or over have had persistent knee pain lasting for at least one week during a month period [266, 267, 270]. Although the mechanism of knee pain is not fully clear, many factors have been shown to be associated with knee pain including demographic, structural, genetic and central factors (such as pain-coping strategies or beliefs about knee pain) [95, 228, 271-275].

Factors outside knee joint structures appear to play a role in pain. Previous twin and sibling pair studies have demonstrated that genetic factors are involved in the pathogenesis of pain, with heritability estimates of approximately 50% for different pain traits [210, 276]. In addition, recent candidate gene studies have investigated gene polymorphisms associated with pain sensitivity [277-280], some of which can discriminate those with painful OA from those without pain. In a previous cross-sectional report we found a higher prevalence of knee pain in people with at least one parent undergoing a TKR for severe primary knee OA at baseline compared with controls, which was independent of structural factors, suggesting a possible role of genetic factors in knee pain [207]. So far, there are no studies examining if people with a family history of knee OA have an increased risk of knee pain over time. The aims of this study were, therefore, to describe whether offspring of people who had at least one parent with TKR for severe primary knee OA would have an increased risk of worsening knee pain over 8 years as compared to controls with no family history of knee OA.

6.2 Materials and methods

6.2.1 Participants

This study was carried out in southern Tasmania in the capital city of Hobart. The initial measurements were taken from June 2000 to December 2001, a total of 372 participants (186 offspring and 186 controls) aged 26 to 61 years (mean age of 45 years) were enrolled. The phase two was conducted 2 years (range 1.8-2.6 years) later, 326 participants (162 offspring and 164 controls) were traced. The phase three was conducted at 10 years (range 9.1-11.4 years), 219 participants (115 offspring and 104 controls) aged 36 to 71 years were included (Figure 6-1). Participants were selected from two sources, as described previously [207, 217, 219]. Half of the participants were the adult children (offspring) of participants who had had a TKR performed for primary knee OA at any Hobart hospital from 1996-2000. This diagnosis was confirmed by reference to the medical records of the orthopaedic surgeon and the original radiograph where possible. The other half were controls selected at random from the state Electoral Roll (2000), without a history of knee OA in either parent. Participants from either group were excluded on the basis of contraindication to MRI (including metal sutures, presence of shrapnel, iron filing in eye, and claustrophobia) and common rheumatoid diseases (rheumatoid arthritis and inflammatory arthritis). This study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee and all participants provided informed written consent.

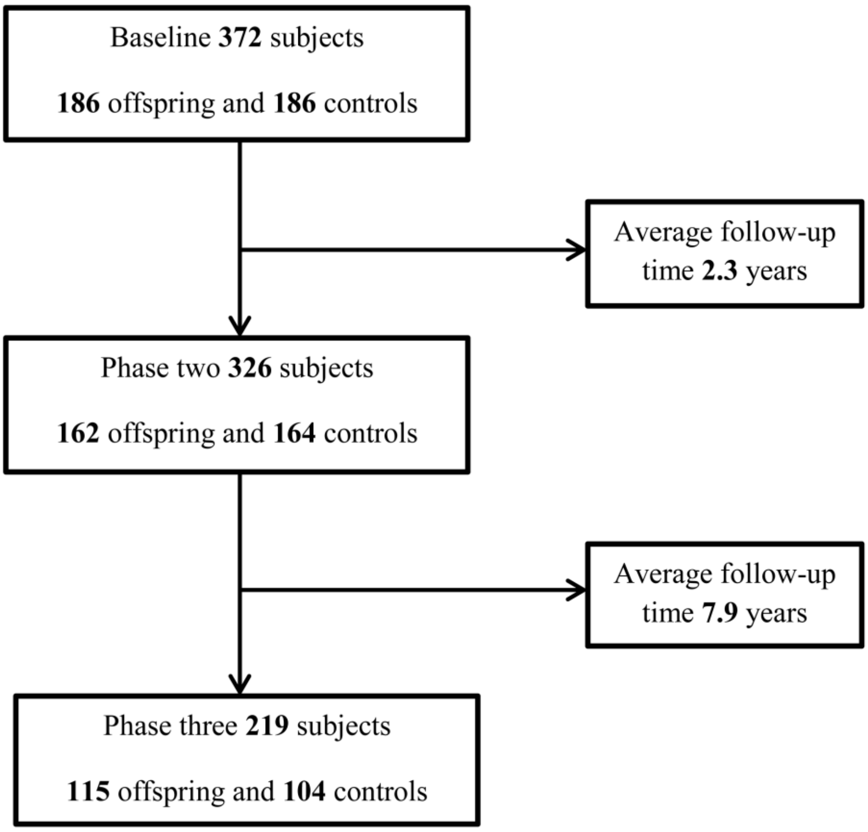

Figure 6-1 Participants inclusion diagram.

6.2.2 Knee pain

Knee pain was only assessed by self-reported pain questionnaire (yes or no pain) at baseline and was defined as pain for more than 24 hours in the last 12 months, or daily pain on more than 30 days in the last year. The WOMAC was utilized to assess knee pain at 2 years and 10 years. The subscales of the WOMAC which consists of five items (walking on flat surface, going up/down stairs, at night in the bed, sitting/lying and standing upright) with a 10-point scale ranging from 0 (no pain) to 9 (most severe pain) were used for this study [281]. Each item was summed to produce a total pain (0-45) score with higher scores indicating greater pain. A total of score of 1 or greater was considered as presence of knee pain at 2 years and 10 years.

6.2.3 Anthropometrics

Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707) calibrated using a known weight at the beginning of each clinic. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI (kg/m^2) was calculated.

6.2.4 Radiographs

A standing anteroposterior semiflexed view of the right knee was performed at baseline in all participants and scored individually using the Altman atlas for osteophytes and JSN as previously described [218]. The presence of medial or lateral tibiofemoral JSN or osteophytes was defined as any score of 1 or greater in that compartment, and 1 or greater in either for whole tibiofemoral JSN or osteophytes.

6.2.5 Magnetic resonance imaging

An MRI of the right knee was performed with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH, US) using a commercial transmit-receive extremity coil at 2 years. As previously described [40, 218], a T1-weighted fat suppression three-dimension gradient recall acquisition and T2-weighted fat saturation two-dimensional fast spin echo acquisition were used. Cartilage defects were graded for medial tibial, medial femoral, lateral tibial, lateral femoral, and patellar sites using 0-4 point scale, as previously described [217], and the scores at these five sites were summed to create a total score of cartilage defect. The presence of any cartilage defect was defined as a score of greater than 1 at any site. Effusion was assessed in the supra-patellar pouch on T2-weighted MR images using 0-3 point scale [282], the presence of effusion was defined as a score of greater than 1. BMLs were assessed on T2-weighted MRI and defined as areas of increased signal adjacent to the subcortical bone, as previously described [40]. The maximum area (cm^2) of the lesion of different sites was measured, and the BML with the largest size was recorded if more than one lesion was present at the same site. The presence of BML was defined as a score of greater than 0 at any site. As previously described [216, 227], meniscal tears and extrusion were evaluated within 6 defined regions (anterior horn, body, and posterior horn of each of the medial and lateral tibiofemoral compartment) using 0-2 point scale. Meniscal tears and extrusion are often correlated and to ensure the least loss of participants [227, 232, 283], any meniscal pathology was created to be a dichotomous variable with a combination of them together in this study. The presence of meniscal pathology was defined as a score of 1 or greater of meniscal tears or extrusion at any site.

6.2.6 Statistical methods

Knee pain was assessed at baseline by simple questionnaire, not by WOMAC questionnaire, so change in WOMAC knee pain as the main outcome was only available from phase two to three. Change in WOMAC was calculated as (phase three value - phase two value) for total pain as well as each subscale. The smallest detectable difference for the WOMAC knee pain score was calculated to be 0.8 for our population [284], so an increase in score of 1 or greater was defined as the cut-off for worsening knee pain. Subscale-specific knee pain was defined as knee pain within the same subscale (for example, knee pain while walking on flat surface and change in knee pain while walking on flat surface). T-tests and Chi-square tests were used to compare differences in means and percentage where appropriate. Mann-Whitney U-test was used to compare absolute change in knee pain between offspring and control group. Logistic regression modelling was used to assess the potential relationships between the status of participants (offspring or controls) and change in knee pain (increase versus no increase) over 8 years, after adjustment for age, sex, BMI, smoking history and knee pain at baseline. Further adjustment for structural factors of relevance to pain in this cohort was also performed. Inverse probability weighting was used to examine whether loss to follow-up biased our results. P values less than 0.05 (2-tailed) or 95% CIs not including the "1" point were regarded as statistically significant. All statistical analyses were performed using SPSS Statistics (version 20; Chicago IL).

6.3 Results

At 10 years of follow-up, a total of 219 participants comprising 115 offspring and 104 controls completed the study. Figure 6-1 describes the study population. 207 (107 offspring and 100 controls) had complete WOMAC pain score information at phase two and phase three. There were no statistically significant differences in characteristics of participants between the participants included in the current study and those lost to follow up except for history of smoking (42% versus 59%).

The characteristics of included participants are presented in Table 6-1. Offspring were heavier than controls, and had a higher percentage of smokers. No significant differences were observed between the two groups in cartilage defects, meniscal pathology, effusion, BMLs and ROA. At baseline, offspring had a higher prevalence of knee pain, no significant difference at phase two; and a significantly higher prevalence at phase three compared to controls. In unadjusted analyses, offspring had a higher knee pain scores while walking and climbing stairs at phase two, and had a higher total knee pain score which approached but did not reach significance. Pain scores in total knee pain and each subscale were consistently greater in magnitude than controls at phase three.

Table 6-1 Characteristics of participants*

Parameter	Offspring	Controls	*P*
Age, years	47.55 ± 6.56	47.65 ± 6.09	0.909
Female (%)	55	61	0.386
Height, cm	169.37 ± 8.83	167.85 ± 8.92	0.220
Weight, kg	**81.64 ± 17.90**	**75.93 ± 15.04**	**0.014**
BMI, kg/m^2	**28.36 ± 5.37**	**26.93 ± 4.99**	**0.049**
Previous knee injury† (%)	15	23	0.116
Ever smoking† (%)	**50**	**33**	**0.008**
Radiographic OA† (%)	17	18	0.865
Any BML (%)	68	60	0.224
Any cartilage defect (%)	60	50	0.149
Any meniscal pathology (%)	20	20	0.995
Prevalent knee pain‡ (%)			
Phase one	**45**	**20**	**<0.001**
Phase two	56	54	0.764
Phase three	**74**	**54**	**0.002**
Knee pain at phase two			
Pain on flat surface	**0.64 ± 1.51**	**0.24 ± 0.77**	**0.016**
Pain on stairs	**1.52 ± 2.20**	**1.00 ± 1.48**	**0.044**
Pain in bed at night	0.51 ± 1.36	0.31 ± 0.97	0.211
Pain sitting	0.43 ± 1.05	0.27 ± 0.80	0.211
Pain standing	0.65 ± 1.56	0.35 ± 0.91	0.091
Total pain	3.48 ± 5.97	2.17 ± 3.94	0.063
Knee pain at phase three			
Pain on flat surface	**1.16 ± 1.70**	**0.61 ± 1.09**	**0.005**
Pain on stairs	**1.82 ± 2.23**	**1.11 ± 1.70**	**0.008**
Pain in bed at night	**1.31 ± 2.04**	**0.60 ± 1.19**	**0.002**
Pain sitting	**1.10 ± 1.69**	**0.56 ± 1.08**	**0.003**
Pain standing	**1.21 ± 1.69**	**0.63 ± 1.09**	**0.003**
Total pain	**6.60 ± 8.39**	**3.49 ± 5.51**	**0.001**

Bold denotes statistically significant result; BMI, body mass index; OA, osteoarthritis; BML, bone marrow lesion;
*Mean ± SD except for percentages; P values determined by t-test or Pearson Chi-square test (where appropriate);
†Variables were measured at phase one;
‡Knee pain was measured by questionnaire at phase one (yes or no pain), and defined as a total of score of 1 or greater at phase two and phase three through WOMAC;

Appendices 6-1 Change in WOMAC scores between offspring and controls over 8 years

	Offspring (n=115)	Controls (n=104)	*P*
Stiffness	1.31 ± 0.33	0.74 ± 0.39	0.262
Function	5.54 ± 1.46	4.13 ± 1.72	0.531
Total WOMAC	10.53 ± 2.34	6.42 ± 2.66	0.246

Changes in knee pain over 8 years between offspring and controls are presented in Figure 6-2. There were increases over time in both groups, but these were greater in the offspring for all categories (both changes in magnitude and increases) apart from knee pain on a flat surface and climbing stairs. No significant difference was found in change in total WOMAC and other subscales of the WOMAC (stiffness and physical function) (Appendices 6-1).

Table 6-2 describes the associations between offspring-control status and risk of any increase in knee pain. In univariable analyses, offspring status was associated with an increase in total knee pain and all subscales with the exception of knee pain on a flat surface. In multivariable analyses, the associations between the status of participants and increases in knee pain remained statistically significant after adjustment for age, sex, BMI, smoking history and baseline knee pain. After further adjustment for cartilage defects, BMLs, meniscal pathology and effusion, these associations persisted for total knee pain and all subscales apart from knee pain on a flat surface and standing of which the latter was of borderline significance. Intriguingly, a trend to a dose-response relationship was found between the number of parent with TKR and change in total knee pain and each subscale. Numerically greater odds ratios (ORs) were seen in those people with two parents undergoing TKR than those offspring with one parent with TKR (Table 6-3). Consistent results were observed after re-analyses of data using inverse probability weighting.

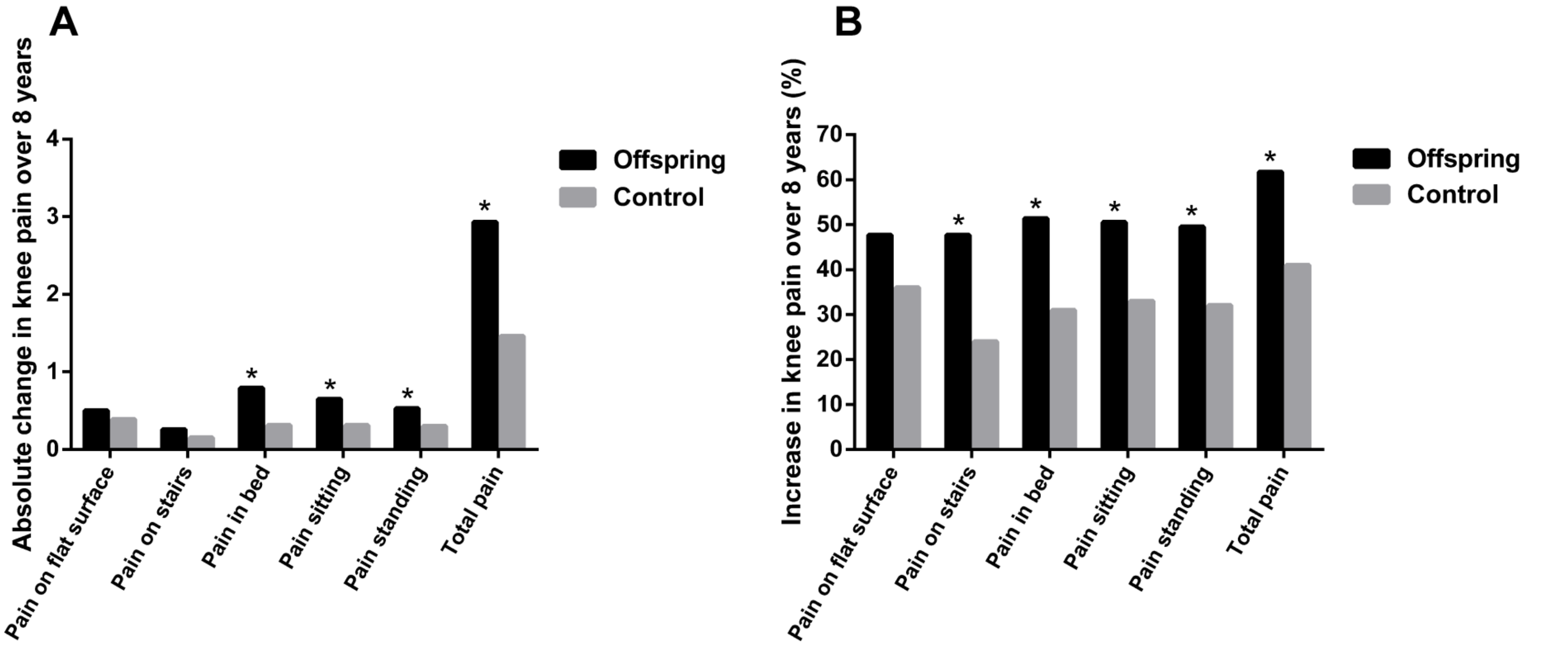

Figure 6-2 Changes in knee pain between offspring and control over 8 years. (A) Absolute changes in knee pain (Mean scores); (B) The participants with an increase in knee pain scores of 1 or greater (%). Offspring had greater changes in knee pain scores and higher proportion of worsening knee pain as compared to the controls. *P< 0.05 compared with control.

Table 6-2 Association between offspring-control status and any increase in knee pain over 8 years

Outcome	Univariable analysis	Multivariable analysis†	Multivariable analysis‡	Multivariable analysis‡‡
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Pain on flat surface	1.59 (0.91, 2.77)	1.74 (0.96, 3.16)	1.77 (0.93, 3.35)	1.70 (0.89, 3.25)
Pain on stairs	**2.78 (1.54, 5.04)**	**3.46 (1.84, 6.53)**	**3.39 (1.71, 6.73)**	**3.30 (1.66, 6.60)**
Pain in bed at night	**2.27 (1.29, 3.99)**	**2.41 (1.31, 4.41)**	**2.51 (1.32, 4.78)**	**2.46 (1.29, 4.71)**
Pain sitting	**1.99 (1.14, 3.49)**	**2.20 (1.20, 4.05)**	**2.04 (1.06, 3.90)**	**1.95 (1.01, 3.74)**
Pain standing	**2.05 (1.16, 3.60)**	**2.31(1.26, 4.24)**	**1.90 (1.00, 3.61)**	1.85 (0.97, 3.52)
Total pain	**2.32 (1.33, 4.05)**	**2.46 (1.35, 4.51)**	**2.25 (1.19, 4.26)**	**2.16 (1.14, 4.12)**

Bold denotes statistically result; OR odd ratio; CI confidence interval.

†Adjusted for age, sex, body mass index, smoking, baseline pain;

‡Further adjusted for knee injury, radiographic osteoarthritis, any cartilage defect and any bone marrow lesion;

‡‡Further adjusted for any meniscal pathology and effusion.

Table 6-3 Association between the number of parent with TKR and any increase in knee pain

The number of parent with TKR	Univariable analysis	Multivariable analysis†
	OR (95% CI)	OR (95% CI)
Pain on flat surface		
0	Ref.	Ref.
1	1.64 (0.93, 2.88)	1.68 (0.87, 3.23)
2	1.81 (0.35, 9.41)	2.05 (0.32, 13.25)
Pain on stairs		
0	Ref.	Ref.
1	2.85 (1.56, 5.20)	3.25 (1.62, 6.53)
2	3.21 (0.61, 16.95)	4.59 (0.69, 30.77)
Pain in bed at night		
0	Ref.	Ref.
1	1.99 (1.13, 3.53)	2.27 (1.18, 4.38)
2	10.78 (1.21, 96.10)	12.43 (1.22, 126.91)
Pain sitting		
0	Ref.	Ref.
1	1.98 (1.12, 3.50)	1.90 (0.98, 3.69)
2	4.12 (0.72, 23.66)	2.88 (0.40, 20.74)
Pain standing		
0	Ref.	Ref.
1	2.03 (1.15, 3.60)	1.78 (0.93, 3.41)
2	4.31 (0.75, 24.78)	3.63 (0.52, 25.10)
Total pain		
0	Ref.	Ref.
1	2.20 (1.25, 3.86)	2.15 (1.12, 4.14)
2	2.81 (0.49, 16.05)	2.32 (0.35, 15.32)

TKR, total knee replacement; OR, odd ratio; CI, confidence interval; Ref, reference group (control);

†Adjusted for age, sex, body mass index, smoking, baseline pain, knee injury, radiographic OA, any cartilage defect, any bone marrow lesion, any meniscal pathology, and effusion.

6.4 Discussion

This study found that offspring of those with severe knee OA had an increased risk of both prevalent pain and worsening knee pain over 8 years as compared to controls who had no family history of OA, and this relationship persisted after adjustment for potential confounding factors and for joint structural abnormalities of relevance to pain. This implies that the genetic contribution to knee pain may be mediated through factors outside the joint possibly pain processing.

To date, there have been limited studies investigating the role of family history of knee OA on knee pain. Previously we reported, in a larger sample, that offspring had a higher prevalence of knee pain as assessed by simple questionnaire at baseline [207]. Consistent with this, the current study also found significant differences in knee pain scores (both prevalence and severity) assessed by the WOMAC at 10 years. Surprisingly, there was no difference in the overall prevalence of knee pain at 2 years although there were higher pain scores while walking on flat surface and climbing stairs. This variation may reflect the use of different questionnaires or more likely implies a greater effect on incident pain.

Pain experience in knee OA is a complex feature, the underlying aetiology of knee pain is multifactorial [95]. Many earlier studies have shown that older age [266, 273, 274], female sex [266, 285, 286], previous knee injury [287, 288] and smoking [288, 289] appear to be important risk factors for developing knee pain. Also, overweight or obesity shows a causal relationship with the development of knee pain and knee OA [288, 290-292]. Despite structure-symptom discordance in radiographs, knee structural abnormalities on MRI have been consistently associated with knee pain [26, 95]. The present study found greater weight, more former smokers and less knee injury in the offspring group; however, in this study, after adjustment for these factors and structural factors, the association with knee pain remained largely unchanged, suggesting that the differences cannot be explained by these factors.

Genetic predisposition to the development of knee pain appears important. The findings of present study that offspring have elevated risk of worsening knee pain, with around two-fold higher risk than controls for total knee pain as well as subscales suggest underlying genetic components in worsening knee pain. These results are consistent with previous twin studies [293] and earlier reports from our group which found the heritability of knee pain was higher in sib pairs [210]. However, whether family factors such as pain-coping strategies, traditions and beliefs about knee pain have a role is unclear [274, 294].

OA-related pain is a complex integration of sensory, affective, and cognitive processes [295], driven by both nociceptive and neurobiological mechanisms [296], each of which involves a number of proteins throughout the peripheral and central nervous systems, whose effects have been shown to be affected by the interplay between environmental and genetic factors [175, 293]. Several studies have documented that genetic mutations can confer hypersensitivity or insensitivity to pain stimuli [297, 298]. Therefore, it is plausible that genetic factors may have a role in the pain sensitivity. Recent studies have attempted to examine the relationship between genes associated with pain sensitivity and OA-related pain [277-279]. One study identified a genetic variant (Val158Met) in the catechol-O-methyltransferase (COMT) gene involved in pain sensitivity to be associated with hip pain among those with hip OA [277] but this was not confirmed in independent cohorts [280]. Subsequently, Valdes *et al*. [278] found allelic variation in the lle585Val variant for the gene encoding transient receptor potential cation channel, subfamily V, member 1 (TRPV1) was able to discriminate those with and without painful OA. In another study, the single nucleotide polymorphism in the proprotein convertase subtilisin/kexin type 6 (PCSK6) gene also showed a strong protection against pain in those with knee OA [279]. When combined with the lack of clear replication for radiographic OA at this point in time, these studies imply that specific genes and/or other multiple extra-articular factors may be more important in the pathogenesis of knee pain than radiographic OA [95, 299], and that higher risk of worsening knee pain for offspring might be attributed to a difference in pain processing in the offspring.

The current study has several potential limitations. First, the proportion followed up was 59% at 10 years, so participants lost to follow-up may lead to bias; however, re-analyses of data using inverse probability weighting did not change any of the results, indicating robust results. Second, knee pain was measured using different methods, simple questionnaire at baseline and WOMAC at 2 years and 10 years so we are unable to directly compare baseline with later phases. Both methods to assess pain may result in recall bias due to variation in reporting of pain [300], especially for offspring with family history of knee OA. Third, knee pain may result from other musculoskeletal diseases or other sites of the body [266, 301]; however, we have not screened for these conditions and did not evaluate pain in other sites. Fourth, pain is a complex feature with multiple factors determining the report of pain. In addition to genetic and structural factors, socio-economic factors such as educational level, occupational stress, satisfaction, and job loss, as well as psychosocial factors such as anxiety and depression also have a role in pain perception [302]. However, these factors were not measured in this study, and therefore we were unable to assess if these factors affect our results. Furthermore, offspring are more likely to be affected by family factors such as pain-coping strategies, traditions and beliefs about knee pain; however, these factors were not available in this study, so we cannot evaluate the influence of these factors. Lastly, several variables such as ROA were not measured at 2 years because of the perceived insensitivity of X-ray to detect radiological changes over this short period, it is unlikely that radiological changes would be different during that period.

In summary, this longitudinal study identified offspring of people with a TKR for severe primary knee OA have an increased risk of worsening knee pain compared with controls and this relationship is independent of knee structural factors, suggesting that genetic factors may be involved in the pathogenesis of knee pain in middle life.

Chapter 7: Associations between fat mass and multi-site pain: a 5-year longitudinal study

7.1 Introduction

Musculoskeletal pain is common affecting people of all ages (particularly in the elderly with prevalence estimates of 10%-50%) and often occurs at multiple sites [303-308]. A recent study showed that three quarters of those with musculoskeletal pain have pain at multiple sites [309]. Evidence from previous studies demonstrated that pain at multiple sites is associated with poorer physical and psychological health, worse health-related quality of life, and disability when compared to people with pain at a single-site [310-314].

MSP, often defined as number of painful sites of two or more, is complex and multi-factorial, and the underlying mechanisms remain unclear. Risk factors for MSP include older age [274, 304, 315], female gender [274, 306, 315], physical inactivity [305, 308], lower educational attainment [274, 309], unemployment [316, 317], psychological distress [305, 310, 315] and genetic factors [318, 319], although the evidence is inconsistent and may vary by site. Moreover, BMI or weight can predict the development of pain at different sites, indicating a possible causal relationship between overweight or obesity and pain [320, 321]. It has long been assumed that the mechanism by which overweight or obesity contributes to pain is due to increased physical loading; however, there is accumulating evidence to suggest a role of metabolic factors as obesity is linked to a low level of systemic chronic inflammation. Recent evidence suggests that this may be related in turn to pain [322]. Additionally, loading is insufficient to explaining pain occurring at non-weight bearing sites such as the hand [155, 308].

BMI is frequently used to measure and classify obesity in the majority of studies investigating the association between obesity and pain; however, it cannot adequately disaggregate the specific components of body composition which have been found to have different roles in the pathogenesis of musculoskeletal diseases [323]. Fat mass is associated with markers of inflammation in overweight or obese individuals. More recently, studies have reported a specific detrimental effect of fat mass for low back pain [324] and foot pain [325, 326]. Few studies have examined the relationship between fat mass and MSP. Only limited information is available in two cross-sectional studies [323, 327] which reported a positive association fat mass with MSP. Such cross-sectional studies cannot determine whether MSP precedes obesity or vice versa. Also, the studies did not adjust for potential confounders including socio-demographic, physical activity and psychological factors. Therefore, the aim of this study was to describe cross-sectional and longitudinal associations between fat mass and MSP in a population-based sample of older adults, and explore the mechanisms underlying this relationship.

7.2 Patients and Methods

7.2.1 Participants

This study utilized data from the TASOAC, a longitudinal, observational population-based study. A total of 1,099 participants aged 50–80 years (mean age 63 years) were randomly selected using computer generated random numbers from the electoral roll in Southern Tasmania (population 229,000), with an equal number of men and women. Baseline measures (Phase 1) were conducted in 2002. The follow-up measures were taken approximately 2.6 years (Phase 2, n=875) and 5.1 years (Phase 3, n=768) later. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, and all participants provided written informed consent.

7.2.2 Primary outcome measurement: pain at multi-sites

The location of sites at which the participants experienced pain was self-reported at baseline, phase 2 and phase 3. Participants were asked whether they had pain (yes/no) in the following sites: neck, back, hands, shoulders, hips, knees or feet. The total number of painful sites (range 0 to 7) was categorised into four groups (no pain, 1-2, 3-4 and 5-7 painful sites) according to the number of painful site groups with approximately equal numbers of participants reporting one or more painful sites [328].

7.2.3 Primary exposure measurement: body composition

Body composition was measured at baseline, phase 2 and phase 3 by dual-energy X-ray absorptiometry (DXA) using a Hologic Delphi densitometer (Model: Hologic Discovery QDR; Software: Apex system software 2.4.2; Manufacturer: Hologic, Waltham, MA, US), which is a quick, non-invasive scan and the gold standard in body composition. A DXA machine works through producing two very low dose x-ray beams, each with different energy levels. Differences in densities of each tissue type lead to different levels of absorption which allow the DXA to calculate their relative masses [329]. Fat mass index (FMI) was calculated as: FMI=fat mass/height^2.

7.2.4 Potential covariates measurements

7.2.4.1 Anthropometrics

Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707) calibrated using a known weight at the beginning of each clinic. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Height and weight were measured at each time-point and were then used to calculate BMI (kg/m^2).

7.2.4.2 Physical activity

Physical activity was assessed at baseline, phase 2 and phase 3 as steps/day determined by pedometer (Omron HJ –003 & HJ–102, Omron Healthcare, Kyoto, Japan), as previously described [330]. Briefly, participants were instructed to wear a pedometer for seven consecutive days and to record the number of steps each day and the duration and type of physical activity for any activities in which the pedometer could not be worn (for example, swimming). This was repeated six months later to account for seasonal variation. Mean steps/day was calculated as the average of the days worn at both time points.

7.2.4.3 Emotional problems

Emotional problems were assessed at baseline using short form-8 Health Survey by asking the question: ‘how much have you been bothered by emotional problems during the past four weeks, such as feeling anxious, depressed or irritable?’. Responses included ‘not at all’, ‘very little’, ‘moderately’, ‘quite a lot’ and ‘extremely’. The presence of emotional problems was defined as a response of ‘very little’ or more.

7.2.4.4 Employment

Employment status at baseline was self-reported and collapsed into two categories: employed (full/part-time) and no paid employment (home duties, student, sole parent/disability pension, retired or unemployed).

7.2.4.5 Education level

Participants reported the highest education level they had completed at baseline, which was collapsed into three categories: low = school only, medium = trade/vocational certificate, high = university level or above.

7.2.5 Statistical analysis

Mean $\pm$ SD and percentages were respectively used to express the continuous and categorical variables, as noted. ANOVA and ordinal $\chi 2$ test (Kruskal-Wallis test) were used to test if there was a trend of mean of each continuous and categorical variable across pain groups. Longitudinal data were analysed using mixed-effects models that take repeated observations on participants into account and use all data on participants. To assess associations of total fat mass, FMI and BMI with MSP, mixed-effect models with random intercepts for participants were used, without and with adjustment for factors, such as age, sex, height, smoking history, physical activity, emotional problems, education level and employment. Additionally, we analysed the associations of total fat mass, FMI and BMI with pain at each site pain after adjusting for the same factors to explore the mechanisms underlying the association. We tested for interaction between each study factor (total fat mass, FMI and BMI) and follow-up time, but no significant interactions were found. To compare odds ratios (ORs), fat mass, FMI and BMI were standardised by dividing by the corresponding SD; therefore, all ORs represent the odds of pain associated with one SD increase in total fat mass, FMI or BMI. All statistical analyses were performed using Stata V.12.1 for windows (StataCorp, College Station, Texas, US). P values less than 0.05 (two-tailed) were regarded as statistically significant.

7.3 Results

The participants were on average 63 years old, 51% female and had a mean BMI of 27.9 kg/m^2 at baseline. There were 768 participants participating in follow-up over 5.1 years with three examinations contributing 2,742 person-examinations. Table 7-1 describes the characteristics of participants at each examination. Weight, BMI, fat mass and FMI increased by a small amount over 5.1 years, but physical activity decreased markedly.

The baseline characteristics of participants by number of painful sites are presented in Table 7-2. A total of 1,086 participants who had complete data on fat mass and pain were included into the analyses. 87% of participants had pain in at least one site, with 28% having pain at one or two sites, 28% having pain at three or four sites, and 31% had pain at five or more sites. Female sex, higher weight, BMI, fat mass and FMI, lower levels of physical activity, having emotional problems, being unemployed and having lower education level were associated with reporting pain at a greater number of painful sites.

Table 7-1 Characteristics of participants at each examination*

Characteristics	**Baseline (n=1099)**	**Phase 2 (n=875)**	**Phase 3 (n=768)**	**P value**
Age, years	63.0±7.5	65.3±7.3	67.1±7.0	<0.001
Female (%)	51	49	50	0.648
Height (cm)	167.0±9.0	167.0±9.0	166.6±9.0	0.549
Weight (kg)	77.9±15.0	78.1±14.8	78.1±14.8	0.922
Body mass index (kg/m^2)	27.9±4.8	28.0±4.8	28.1±4.8	0.594
Fat mass (kg)	28.3±8.7	28.2±9.0	28.4±8.7	0.954
Fat mass index (kg/m^2)	10.3±3.6	10.3±3.7	10.4±3.7	0.854
Ever smoking (%)†	51	NA	NA	NA
Physical activity (steps/day)	8614.9±3354.8	7405.2±3358.0	6828.4±3179.8	<0.001
Emotional problems (%)†	64	NA	NA	NA
Employed (%)†	39	NA	NA	NA
Education level (%)†				NA
School only	56	NA	NA	
Vocation training	32	NA	NA	
University or higher	11	NA	NA	
Multi-site joint pain (%)				0.001
No pain	13	19	17	
1-2 sites	29	29	31	
3-4 sites	28	28	27	
5-7 sites	31	24	26	

*Values are the Mean±SD except for percentages;

†Variables were measured at baseline.

Table 7-2 Descriptive characteristics of participants at baseline, by number of painful joints*

	Number of painful sites				P value
	0 (n=137)	1-2 (n=310)	3-4 (n=303)	5-7 (n=336)	
Age, years	62.2±7.2	63.6±7.7	62.4±7.2	63.3±7.7	0.676
Female (%)	45	48	52	57	0.005
Height (cm)	167.4±9.0	167.6±9.4	167.4±8.6	165.9±8.8	0.028
Weight (kg)	73.5±12.7	77.3±15.6	79.2±15.3	79.2±14.8	<0.001
Body mass index (kg/m^2)	26.2±3.9	27.4±4.5	28.2±4.5	28.8±5.3	<0.001
Fat mass (kg)	25.0±7.1	27.5±8.5	28.8±8.1	30.0±9.5	<0.001
Fat mass index (kg/m^2)	9.1±3.0	9.9±3.4	10.4±3.3	11.1±4.1	<0.001
Ever smoking (%)	46	51	49	55	0.104
Physical activity (steps/day)	9495.1±3579.4	8759.4±3274.9	8560.0±3258.4	8078.2±3341.0	0.001
Emotional problems (%)	53	56	62	70	<0.001
Employed (%)	50	41	42	31	<0.001
Education level (%)					<0.001
School only	49	55	56	61	
Vocational training	35	31	30	34	
University or higher	16	14	13	5	

*Values are the Mean±SD except for percentages; ANOVA and ordinal $\chi2$ test (Kruskal-Wallis test) were used to test if there was a trend of mean of each continuous and categorical variable (increase or decrease) across pain groups.

Figure 7-1 shows the association of fat mass and BMI with number of painful sites at each examination. Fat mass and BMI increased with each category of MSP. However, there was no statistically significant increase in fat mass or BMI over 5.1 years within any pain category. Similar results were seen for FMI (Figure B).

The associations of fat mass, FMI and BMI with MSP are shown in Table 7-3. In univariable analysis, each SD increase in fat mass, FMI, or BMI was associated with increased odds of reporting MSP. The associations were reduced but remained statistically significant after adjusting for age, sex and height, and after further adjusting for smoking history, physical activity, emotional problems, education level and employment.

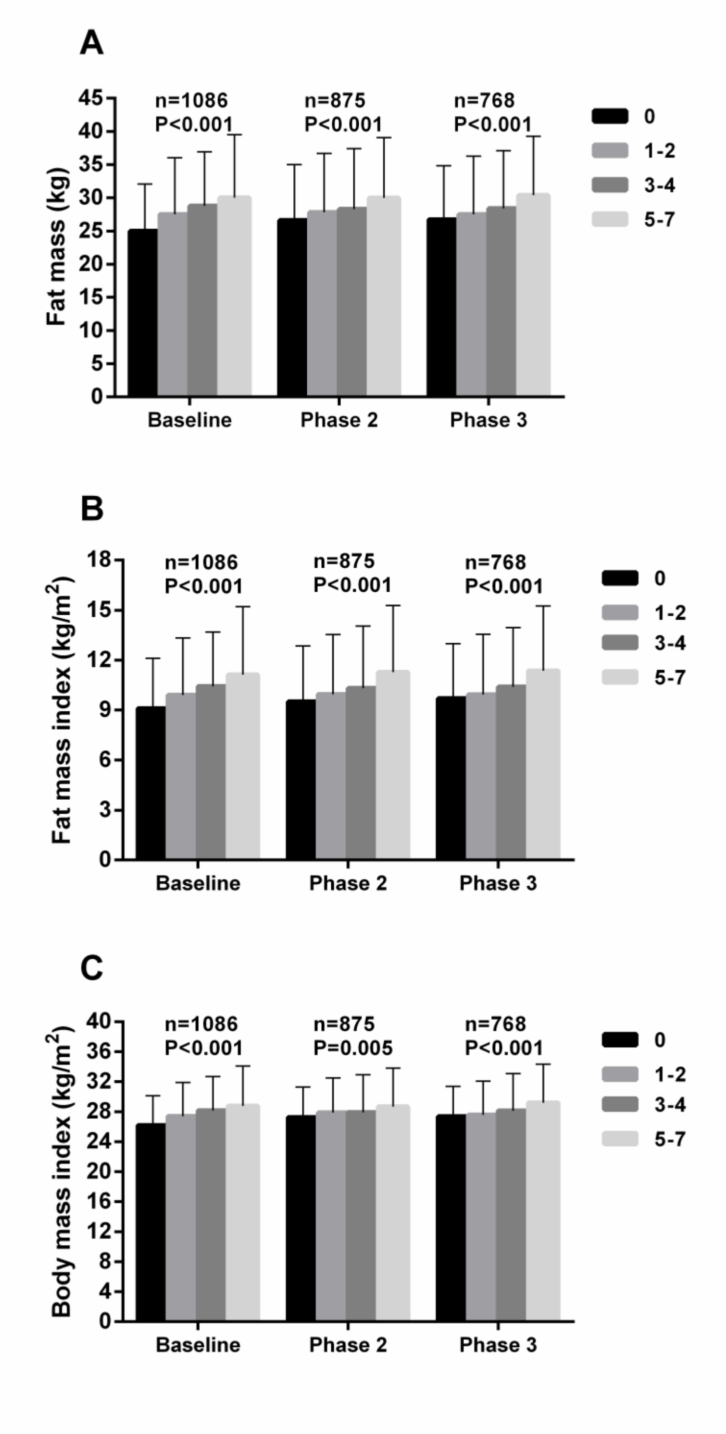

Figure 7-1 Association between fat mass/body mass index and the number of painful sites. Bar graph represents mean value of fat mass/body mass index, and error bars indicate standard deviations. P for trend determined by ANOVA test. (A) Fat mass; (B) Fat mass index; (C) Body mass index.

Table 7-3 Association between fat mass, fat mass index and body mass index and multi-site pain (Number of groups=1086)*

	Univariable		Multivariable†		Multivariable‡	
	OR	95% CI	OR	95% CI	OR	95% CI
Fat mass	**1.10**	**1.06, 1.14**	**1.08**	**1.04, 1.12**	**1.06**	**1.02, 1.10**
Fat mass index	**1.11**	**1.07, 1.14**	**1.09**	**1.05, 1.13**	**1.07**	**1.03, 1.11**
Body mass index	**1.09**	**1.05, 1.12**	**1.08**	**1.05, 1.12**	**1.07**	**1.04, 1.11**

Bold denotes statistically significant result.

*OR (95% CI): odds ratio (95% confidence interval) representing the OR of greater number of painful sites associated with per SD increase in fat mass, fat mass index or body mass index;

†Fat mass adjusted for age, sex and height; fat mass index and body mass index adjusted for age and sex only.

Table 7-4 Association between fat mass, fat mass index and body mass index and site-specific pain (Number of groups=1086)*

	Pain site	Univariable		Multivariable†		Multivariable‡	
		OR	95% CI	OR	95% CI	OR	95% CI
Fat mass							
	Neck	1.18	0.97, 1.45	1.04	0.84, 1.29	1.00	0.80, 1.26
	Back	**1.24**	**1.03, 1.51**	1.17	0.95, 1.44	1.20	0.97, 1.49
	Shoulders	**1.27**	**1.07, 1.51**	1.15	0.95, 1.38	1.08	0.89, 1.31
	Hands	**1.54**	**1.27, 1.86**	**1.37**	**1.12, 1.67**	**1.29**	**1.04, 1.59**
	Hips	**1.53**	**1.27, 1.84**	**1.41**	**1.16, 1.71**	**1.38**	**1.13, 1.70**
	Knees	**1.96**	**1.61, 2.39**	**1.98**	**1.61, 2.44**	**1.99**	**1.59, 2.49**
	Feet	**1.89**	**1.56, 2.28**	**1.79**	**1.46, 2.18**	**1.87**	**1.51, 2.32**
Fat mass index							
	Neck	**1.28**	**1.05, 1.57**	1.05	0.83, 1.33	1.00	0.78, 1.28
	Back	**1.29**	**1.06, 1.57**	1.20	0.95, 1.50	1.22	0.95, 1.56
	Shoulders	**1.39**	**1.16, 1.65**	1.19	0.97, 1.46	1.10	0.88, 1.37
	Hands	**1.73**	**1.43, 2.11**	**1.47**	**1.18, 1.84**	**1.37**	**1.08, 1.73**
	Hips	**1.62**	**1.34, 1.95**	**1.47**	**1.18, 1.82**	**1.42**	**1.13, 1.79**

	Knees	**1.86**	**1.53, 2.27**	**2.07**	**1.64, 2.61**	**2.06**	**1.60, 2.64**
	Feet	**1.95**	**1.61, 2.36**	**1.90**	**1.52, 2.37**	**1.99**	**1.57, 2.53**
Body mass index							
	Neck	1.10	0.90, 1.36	1.09	0.89, 1.34	1.06	0.86, 1.31
	Back	**1.23**	**1.01, 1.50**	**1.23**	**1.01, 1.49**	**1.25**	**1.02, 1.54**
	Shoulders	**1.21**	**1.02, 1.44**	**1.20**	**1.01, 1.42**	1.14	0.95, 1.37
	Hands	**1.48**	**1.22, 1.79**	**1.46**	**1.20, 1.77**	**1.41**	**1.15, 1.72**
	Hips	**1.49**	**1.23, 1.79**	**1.46**	**1.22, 1.76**	**1.45**	**1.20, 1.76**
	Knees	**1.95**	**1.60, 2.38**	**1.94**	**1.59, 2.37**	**1.94**	**1.57, 2.40**
	Feet	**1.77**	**1.46, 2.14**	**1.75**	**1.45, 2.12**	**1.84**	**1.50, 2.26**

Bold denotes statistically significant result.

*OR (95% CI): odds ratio (95% confidence interval) representing the OR of greater number of painful sites associated with per SD increase in fat mass, fat mass index or body mass index;

†Fat mass adjusted for age, sex and height; fat mass index and body mass index adjusted for age and sex only.

‡Further adjusted for smoking history, physical activity, emotional problems, education level and employment;

Table 7-4 presents the associations of fat mass, FMI and BMI with presence of pain at each site. In univariable and multivariable analysis adjusting for the same confounders as for MSP, greater fat mass was associated with greater odds of pain in lower limbs (knees, hips and feet) and hands. Results were similar with FMI and BMI as the outcome, but BMI was also associated with increased odds of back pain. There were no statistically significant associations between measures of fat mass and pain at the neck or shoulders in multivariable analysis.

7.4 Discussion

This study shows that fat mass, FMI and BMI are associated with MSP, pain at weight-bearing sites and hand pain. These relationships are independent of demographic factors, physical activity, psychological health, education level and employment, suggesting that fat mass may play an important independent role in the pathogenesis of MSP. This may reflect a role of systemic inflammatory factors in joint pain as one would not expect to observe significant association of fat mass with hand pain.

The high prevalence of pain at multiple sites is consistent with that reported in previous studies [301, 305, 309, 315, 323, 331-334], and confirms that MSP is extremely prevalent in a community-based older population. However, some prior studies have found greater prevalence of MSP than that found in our study. These discrepancies may be attributed to the difference in the characteristics of the population studied, definition of MSP and number of painful sites assessed. Our results also showed that the prevalence of MSP with more than two painful sites did not change much over time in the whole population with slightly over half at each visit. This suggests that MSP is likely to be relatively stable once established [304].

The findings that fat mass, FMI and BMI are associated with increased risk of pain at multiple sites indicates a substantial effect of fat mass or body weight on the pathogenesis of MSP. Our findings not only add further evidence to support the significant role of fat mass in pain, but extend previous cross-sectional studies to longitudinal analyses with a large dataset. Our results are consistent with previous studies in which BMI was used to examine the association between overweight/obesity and MSP. In a recent longitudinal study performed in the general population, Magnusson *et al.* [321] found that overweight/obesity increased the odds of reporting MSP. Kamaleri *et al.* [310] found a greater number of painful sites reported in those with a higher BMI. However, the specific components of body composition cannot be distinguished in these studies. Currently, there are only two cross-sectional studies investigating the relationship between body composition and MSP. Brady *et al*. [323] reported that fat mass is associated with an increased number of lower body pain sites (low back, knee and foot), and Yoo *et al.* [327] found fat mass to be positively associated with widespread pain.

The potential mechanisms that may link obesity-related pain are most likely physical loading and metabolic effects [308]. Our results showed that fat mass, FMI and BMI are associated with pain at all weight-bearing sites. This is consistent with previous studies reporting associations between fat mass and single-site pain [325, 326, 335]. These suggest a potential involvement of biomechanical mechanisms, as excess loading may result in changes in body mechanics, postures or abnormal gait, thus creating a detrimental biomechanical environment [336]. However, the finding of a significant association of fat mass, FMI and BMI with hand pain indicates a potential role for metabolic effects in the pathogenesis of pain, since physical loading is not adequate to explaining pain occurring at non-weight bearing sites [320, 337]. It has been recognized that adipose tissue is serving as an endocrine organ to produce proinflammatory cytokines and adipokines [338]. An increased level of cytokines and inflammatory markers, such as C-reactive protein (CRP), Interleukins-6 (IL-6), TNF-alpha (TNF-α) and Leptin observed in obese individuals has been reported in prior studies [339, 340]. Recent evidence suggests that inflammation can lead to a lowering of excitation threshold and enhanced responses to suprathreshold stimuli of peripheral nociceptors (peripheral sensitisation) [95], and subsequently developing central nervous system sensitisation with pain hypersensitivity and increased vulnerability to reporting more pain sites [341]. It is therefore possible that individuals with greater fat mass are more likely to have peripheral or central sensitisation in relation to elevated level of systemic inflammation, thereby leading to a greater number of painful sites.

The current study was unable to detect a significant association between fat mass, FMI and BMI, and neck and shoulder pain, suggesting that neck and shoulder pain are not related to fat mass regardless of the mechanism. Consistent with this, Iizuka *et al.* [342] found no association between fat mass and neck and shoulder pain and concludes that neck and shoulder pain may be manifest through muscle dysfunction. This is supported by reported altered muscle activation patterns in patients with neck/shoulder pain with increased activation of upper trapezius and reduced activation of serratus anterior [343]. BMI, but not other measures of fat mass, was associated with back pain. The reasons for this remain unclear. Overall, the magnitude of the effect per SD increase for fat mass, FMI and BMI were similar for all pain sites, suggesting that DXA derived fat mass is not superior to BMI for accounting for musculoskeletal outcomes.

The current study found that the relationship between fat mass and MSP remained significant after adjusting for age, sex, height, physical activity, education level, employment, and psychological distress, suggesting that the associations with fat mass cannot be fully explained by these factors even if they were themselves associated with pain. Previous studies have demonstrated that body fat [344] and pain [345] are substantially influenced by underlying genetic factors, so it is possible that genetic factors may underlie these associations. This is supported by a recent meta-analysis [346], in which pooling of the results from five twin studies on the relationship between obesity and low back pain showed a positive relationship between BMI or weight and low back pain, but the relationship diminished after adjusting for shared genetic factors, suggesting that genetic factor may be mediating the association.

Limitations of our study include the use of a self-reported questionnaire, which was simple (yes/no) and did not include assessment of frequency, severity, and quality of pain. We, therefore, cannot evaluate whether fat mass is associated with intensity and different types of pain. Additionally, assessments were made on only three occasions over 5.1 years – more frequent observations may allow more information on temporal patterns in fluctuations in pain. Another limitation is that the participants were recruited from one center, which may not be generalisable to other populations as previous studies have indicated that special cultural and socioeconomic conditions may determine individual perceptions affecting the report of pain [319, 347]. Furthermore, although socio-demographic and psychological factors were considered in this study, we cannot exclude the influence of unmeasured factors, such as pain coping strategies. Finally, although fat mass is often considered as a surrogate for systemic inflammation, inflammatory markers were not analysed directly in this study. Accordingly, further investigation into the role of inflammatory markers in the pathogenesis of MSP is warranted.

There are several implications raised from the findings of this study. First, overweight/obese individuals with MSP may benefit from weight loss either via exercise, diet or bariatric surgery. Therefore, the general practitioner should introduce weight management programs involving exercise and diet to overweight/obese individuals and encourage them to change their lifestyles to lose weight, although there is a considerable challenge in the maintenance of weight loss in the long-term. Second, the mechanisms by which overweight/obesity contributes to pain may be not only related to increased physical loading, but also elevated systemic inflammation; thus, facilitating potential therapeutic targets for obesity-related pain. For instance, the administration of drugs with pleiotropic actions (anti-inflammatory and those blocking cholesterol biosynthesis, such as statins) may help to attenuate pain induction in clinical setting; this would need to be tested in a future clinical trial.

To sum up, fat mass, FMI and BMI are associated with MSP, pain at all lower limb sites and hand pain, independent of socio-demographic, physical activity and psychological factors, suggesting that obesity appears to be an important factor in the pathogenesis of fat-related MSP. The potential mechanisms underlying the relationship between fat mass and MSP may be via loading and systemic inflammatory factors.

Chapter 8: Pain at sites outside the knee predicts knee cartilage volume loss in elderly people without knee osteoarthritis: a prospective study

8.1 Introduction

Musculoskeletal pain commonly occurs in older people, with knees the most commonly reported painful site. Joint pain is associated with functional limitation and impaired quality of life [14] and the primary reason why people seek help with knee OA [95]. Isolated knee pain is uncommon in the elderly but rather knee pain is typically accompanied by pain at other sites [311, 312, 348]. Compared to single-site pain, MSP is associated with poorer level of physical and psychological health, worse health-related quality of life, and more severe depressive symptoms in both cross-sectional and longitudinal studies [311, 312, 314].

A number of studies have investigated associations between knee pain and development and progression of knee OA [252, 349-351]. There is no evidence for an association between knee pain and progression of radiographic knee OA based on a previous meta-analysis [352]. Previous studies have demonstrated that loss of cartilage, a major hallmark of OA, is predictive of clinically relevant endpoint of knee replacement [17, 27, 28]; however, studies of the associations between knee pain and knee cartilage volume loss are inconsistent [245, 252, 255, 349-351]. In a 2-year longitudinal study, Cicuttini *et al.* [349] reported that knee pain at baseline was associated with greater patella cartilage volume loss. A study with a 4.5-year follow-up reported that knee pain was associated with a higher rate of medial tibial cartilage volume loss [252]. Saunders *et al.* [350] found that knee pain independently predicted lateral but not medial tibial cartilage volume loss in a 2.9-year follow-up study. In addition, people with frequent knee pain had greater medial tibiofemoral cartilage volume loss than those without [351], while Raynauld *et al.* [245] and Wluka *et al.* [255] did not find any associations between knee pain and change in cartilage volume.

One possible explanation for these inconsistent results is that studies to date have not taken pain at other sites into account. This is important as MSP may be due to higher levels of disease activity, such as systemic factors, dysfunction in central pain processing, or genetic factors [353-357], and thus may represent a different phenotype of pain from single-site pain. The prevalence of those with detectable levels of systemic inflammation was not low in the general population [322]. Furthermore, OA is the most common cause of pain in the elderly; therefore, people with MSP most likely represent the disease activity of OA in a general population. Potential risk factors for knee cartilage volume loss reported in previous studies include older age [358], female sex [358], BMI [150], relevant knee structural abnormalities [26] and genetic factors [359]. Studies that attempt to investigate the relationship between MSP and cartilage volume loss should assess the effect of these potential factors. Therefore, this study aimed to determine whether MSP is a predictor of knee cartilage volume loss, and if so, to explore potential mechanisms.

8.2 Patients and Methods

8.2.1 Participants

This study was conducted as part of the TASOAC-a longitudinal, observational population-based study. The cohort consisted of both men and women and was selected from the electoral roll in Southern Tasmania generated by staff of the Tasmanian Electoral office on 31st January 2002 (total number of people on the roll n=229,593) using sex-stratified simple random sampling without replacement. The eligible cohort consisted of registered electors aged 50–80 years (n=61,715, men/women=29,484/32,231). Institutionalised older adults were excluded because TASOAC was designed to study community-dwelling older adults. A total of 2,530 subjects were selected from the roll using 5-year age band information with equal number of men and women. Among them, 395 were deemed unable to participate due to illness or other reasons, and the remainder were contacted via mail by asking whether they would like to participate in the study. Of 2,135 subjects, 1,100 were enrolled in the study and 1,099 attended the first clinic between March 2002 and September 2004 (response rate 57%) at the Menzies Institute for Medical Research, Australia. The follow-up measures were taken approximately 2.6 years (range 1.4–4.8 years) later (n=875) (retention rate 80%). MRI scans were available for only approximately half of the follow-up participants (n=425 of 875). The current study consists of a sample of 394 TASOAC participants who had MRI measures at baseline and follow-up and data on pain at baseline. The study was approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee (Ref. no: H0006488), and all participants provided informed written consent.

8.2.2 Primary outcome measurement

8.2.2.1 Knee cartilage volume

MRI scans of the right knee were performed at baseline and after 2.6 years. Knee cartilage volume was determined by means of image processing on an independent work station using Osiris (University of Geneva) and measured by two trained and blinded observers as previously described [243]. The volumes of individual cartilage plates (medial tibia and lateral tibia) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on a section by section basis. These data were then resampled by means of bilinear and cubic interpolation for the final three-dimensional rendering. The CV for baseline and follow-up cartilage volume measures was 2.1% for medial tibial, and 2.2% for lateral tibial cartilage [243]. Knee femoral cartilage volume was determined by means of image processing on an independent workstation using Cartiscope ™ (ArthroVision Inc., Montreal) , as previously described [245]. The segmentation of the cartilage-synovial interfaces was carried out with the semi-automatic method under reader supervision and with corrections when needed. Cartilage volume was evaluated directly from a standardized view of three-dimensional cartilage geometry as the sum of elementary volumes. The CV was approximately 1.6% for medial femoral and 2.9% for lateral femoral cartilage at baseline and follow-up [245]. The cartilage volume assessment was done for the medial and lateral condyles delineated by the Blumensaat's line. The medial, lateral and total tibiofemoral cartilage volume created for this study were the sum of cartilage volume of corresponding sites. Rates of change in cartilage volume were calculated as: percentage change per annum = [100 ×

((follow-up cartilage volume - baseline cartilage volume)/baseline cartilage volume)/time between two scans in years].

8.2.3 Primary exposure measurement

8.2.3.1 Multiple-site pain

The location of sites at which the participants experienced pain was measured by self-reported questionnaire at baseline. Participants were asked whether they had pain (yes/no) in the following sites at present: neck, back, hands, shoulders, hips, knees or feet. The number of painful sites was summed to create a total number of painful site with a range from 0 to 7, which was then categorised into four groups (non-painful site, 1-2, 3-4, 5-7 painful sites) according to the number of painful site groups with approximately equal numbers of participants reporting one or more painful sites [328]. Number of painful site types was also assessed on a regional basis, with total count of painful upper limb sites created by summing the number of painful upper limb sites (neck, hands and shoulders, range 0–3), and count of painful lower limb sites created by summing number of hip, knees and feet (range 0–3).

8.2.4 Measurement of potential covariates

8.2.4.1 Anthropometrics

Weight was measured to the nearest 0.1 kg (with shoes, socks and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707) calibrated using a known weight at the beginning of each clinic. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI (kg/m^2) was calculated.

8.2.4.2 Physical activity

Physical activity was assessed at baseline as steps/day determined by pedometer. Each participant was instructed to wear a pedometer for seven consecutive days and to record the number of steps each day and the duration and type of physical activity for any activities in which the pedometer could not be worn (for example, swimming). This was repeated six months later to account for seasonal variation. Mean steps/day was calculated as the average of the days worn at both time points.

8.2.4.3 Use of pain medication

Participants were asked to list all medication prescribed by a doctor, and any other over-the-counter medications they had taken in the last two weeks, including dosage and frequency. Medications used for pain relief were extracted from this list, and dichotomised into whether they were used or not (yes/no).

8.2.4.4 Radiographs

A standing anteroposterior semiflexed view of the right knee was performed in all participants and scored individually using the Altman atlas for osteophytes and JSN on a scale of 0-3 [360]. The presence of medial or lateral tibiofemoral JSN or osteophytes was defined as any score of 1 or greater in that site, and 1 or greater in either for whole tibiofemoral JSN or osteophytes. The presence of ROA was defined as any score ≥ 1 for JSN or osteophytes.

8.2.4.5 Cartilage defects

Cartilage defects were assessed at baseline on T1-weighted MR images at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as previously described [247], as follows: grade 0 = normal cartilage; grade 1 = focal blistering and intracartilaginous low-signal intensity area with an intact surface and base; grade 2 = irregularities on the surface or base and loss of thickness < 50%; grade 3 = deep ulceration with loss of thickness > 50%; and grade 4 = full-thickness chondral wear with exposure of subchondral bone. The ICCs ranged from 0.80–0.95 for intra-observer repeatability. The presence of any cartilage defect was defined as a score of ≥ 2 at any site.

8.2.4.6 Bone marrow lesions

BMLs were assessed at baseline on T2–weighted MR images and defined as areas of increased signal adjacent to the subcortical bone at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as previously described [45]. The maximum area (mm^2) of the lesion of different sites was measured, and the BML with the largest size was recorded if more than one lesion was present at the same site. The presence of any BML was defined as a score of greater than 0 at any site. The ICC was 0.97 for intra- observer repeat-ability.

8.2.5 Statistical analysis

T-tests and Chi-square were used to compare differences in means and percentages between the participants included and the rest of cohort where appropriate. ANOVA and ordinal $\chi 2$ test (Kruskal-Wallis test) were used to test if there was a trend of mean of each continuous and categorical variable across pain groups. Cartilage volume (either at baseline or % change) was normally distributed in this sample; therefore, linear regression was used to assess the potential associations between the number of painful sites and cartilage volume loss (% per annum), before and after adjustment for age, sex, BMI, physical activity, pain medication, baseline cartilage volume, cartilage defects, BMLs, JSN and osteophytes. Significant interactions between the number of painful sites and knee ROA were detected, suggesting that the effect of the number of painful site on cartilage volume loss was different in participants with and without ROA. Subgroup analyses according to ROA status were therefore performed. We also performed the analyses on the associations between pain at each specific site and cartilage volume loss using linear regression to explore the mechanisms underlying these associations. Sensitivity analyses were performed using inverse probability weighting to determine whether loss to follow-up biased our results. Multiple comparisons on the results of associations between site-specific pain and cartilage volume loss were controlled using the Hochberg method [224]. All statistical analyses were performed using Stata V.12.1 (StataCorp, US).

8.3 Results

In the current study, 394 of 1,099 participants with MRI measures at baseline and follow-up and pain measures were included. The average follow-up time was 2.6 years (range 1.4–4.8). 705 participants were excluded from this study due to loss to follow-up or no data on the MRI and pain. There were no significant differences in age, sex, BMI, physical activity, pain medication, ROA, BMLs as well as the number of painful sites between the participants included (n=394) and the rest of cohort (n=705), except for a slightly higher prevalence of medial tibiofemoral cartilage defects in those who were not included in this study.

Table 8-1 presents the baseline characteristics of participants by category of number of painful sites. The median number of painful sites was 3 (range 0 to 7) and 87% of participants had pain at least one site. Among them, 115 (29%) reported having pain at one or two sites; 110 (28%) had three or four painful sites and 119 (30%) reported pain at five or more sites. Participants reporting a greater number of painful sites were more likely to be women, have higher BMI, higher reported use of pain medication, and a trend to less physical activity. There were no baseline differences in the proportion of participants with ROA, cartilage defects, BMLs and baseline cartilage volume. Increasing number of painful sites was associated with cartilage volume loss over 2.6 years in the lateral and total tibiofemoral compartments with higher rate of cartilage volume loss in participants reporting pain in greater numbers of painful sites. There was also a tendency towards increased medial tibiofemoral cartilage volume loss as number of painful sites increased (Table 8-1and Figure 8-1).

Table 8-1 Descriptive characteristics of participants at baseline, by number of painful sites*

	Number of painful sites				P value
	0 (N=50)	**1-2 (N=115)**	**3-4 (N=110)**	**5-7 (N=119)**	
Age, years	62.2±7.6	64.6±8.0	62.3±6.3	62.6±7.0	0.676
Female (%)	**44**	**40**	**57**	**72**	**0.005**
Body mass index (kg/m^2)	**26.5±4.4**	**27.1±4.1**	**27.9±4.4**	**28.4±4.8**	**<0.001**
Physical activity (steps per day)	**9849.4±4090.6**	**8677.3±2940.5**	**8588.3±2933.8**	**8486.2±3407.4**	**<0.001**
Any pain medication (%)	**43**	**47**	**56**	**74**	**<0.001**
Radiographic knee OA (%)	61	52	61	61	0.151
Any knee cartilage defects (%)	24	32	31	33	0.176
Any knee BMLs (%)	42	42	45	45	0.583
Cartilage volume at baseline (ml)					
MTF	6.3±1.5	6.4±1.7	6.4±1.5	6.1±1.6	0.324
LTF	7.1±1.7	7.3±1.9	7.3±1.6	6.8±1.7	0.190
TF	13.4±3.1	13.8±3.5	13.7±3.1	13.0±3.3	0.235
Percentage change in cartilage volume (per annum)					
MTF	**-1.3±2.8**	**-1.3±2.3**	**-1.8±2.5**	**-2.0±2.7**	**0.040**
LTF	**-0.9±2.2**	**-1.1±1.7**	**-1.5±2.1**	**-1.8±2.2**	**0.003**
TF	**-1.1±2.3**	**-1.2±1.7**	**-1.7±2.1**	**-2.0±2.0**	**0.004**

Bold denotes statistically significant result; OA osteoarthritis; BMLs bone marrow lesions; MTF medial tibiofemoral compartment; LTF lateral tibiofemoral compartment; TF tibiofemoral compartment;
*Values are the Mean±SD except for percentages; ANOVA and ordinal $\chi 2$ test (Kruskal-Wallis test) were used to test if there was a trend of mean of each continuous and categorical variable (increase or decrease) across pain groups.

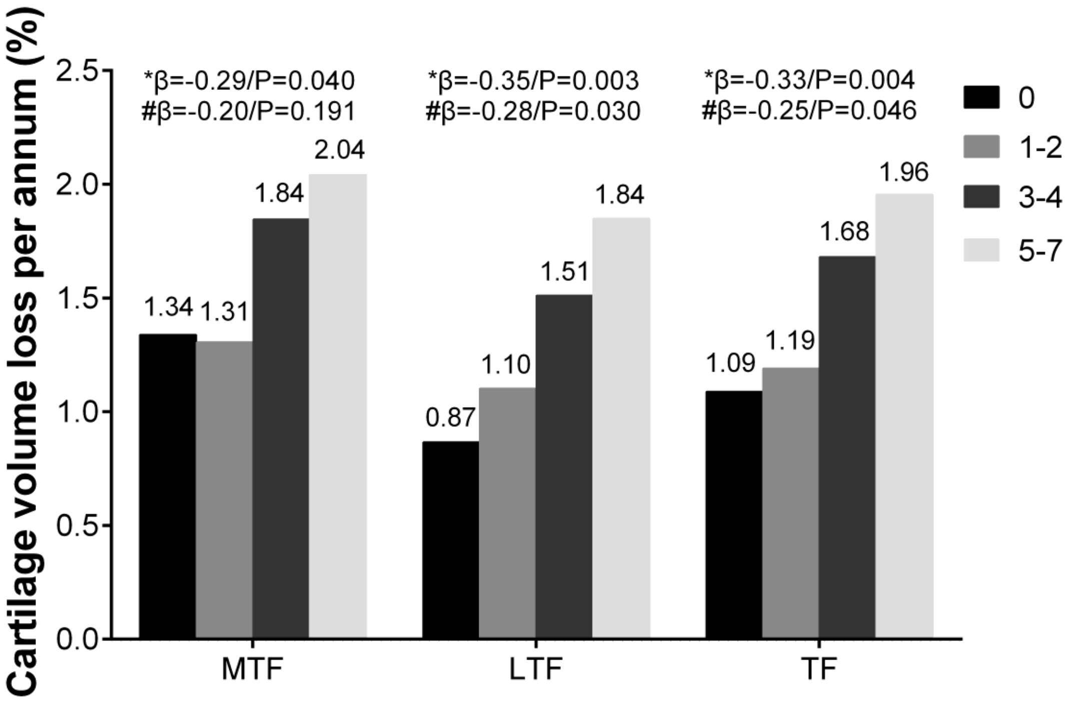

Figure 8-1 The association between number of painful sites and cartilage volume loss in the total sample. With increasing number of painful sites, there is greater annual cartilage volume loss at lateral and total, but not at medial tibiofemoral compartment. The β coefficients and P values are from tests of trend of cartilage volume loss on number of painful sites determined by linear regression. *represents the univariable analysis; #represents multivariable analysis with adjustment for age, sex, body mass index, physical activity, pain medication, baseline cartilage volume, cartilage defects, bone marrow lesions, joint space narrowing and osteophytes for corresponding compartment. MTF medial tibiofemoral compartment; LTF lateral tibiofemoral compartment; TF tibiofemoral compartment.

Figure 8-1 describes the association between the number of painful sites and cartilage volume loss. In unadjusted analyses, cartilage volume loss increased with greater numbers of painful sites in a dose-response manner at all compartments (medial, lateral and total tibiofemoral compartments). After adjustment for age, sex, BMI, physical activity, osteophytes, JSN, cartilage defects and BMLs, these significant associations persisted at lateral (β=-0.28% per annum, 95% CI -0.52%, -0.03%, P=0.030) and total (β=-0.25% per annum, 95% CI -0.49%, -0.01%, P=0.046) tibiofemoral compartments. In the medial tibiofemoral compartment, the magnitude of the effect was only slightly less (β=-0.20% per annum) and did not reach statistical significance (P=0.191), but it showed a similar pattern with lateral and total tibiofemoral cartilage volume loss.

There was a significant interaction between number of painful sites and ROA status for lateral and total tibiofemoral cartilage volume loss, and thus subgroup analysis was conducted by the status of ROA. Table 8-2 and Table 8-3 show the results of association of number of painful sites and lateral and total tibiofemoral cartilage volume loss stratified by ROA status using "non-painful site" as a reference group. The significance of a linear trend was tested using Wald tests and there was a trend if $P<0.05$. For those without ROA, we found that having 1-2, 3-4 and 5-7 painful sites had greater cartilage volume loss in the fully adjusted model, and showed a dose-response relationship for lateral and total tibiofemoral cartilage volume loss (adjusted P for trend=0.002). However, we did not observe any significant associations or dose-response relationship in participants with ROA.

Table 8-2 Association between the number of painful sites and lateral tibiofemoral cartilage volume loss, by ROA status

	No. of pain sites	Annual percentage cartilage volume loss (mean, %)	Univariable		Multivariable†	
			β	95% CI	β	95% CI
Without ROA						
	0	-0.41	Ref.		Ref.	
	1-2	-1.00	-0.59	-1.67, 0.50	-0.73	-1.84, 0.39
	3-4	-1.69	**-1.28**	**-2.43, -0.14**	**-1.37**	**-2.52, -0.22**
	5-7	-1.96	**-1.55**	**-2.67, -0.44**	**-1.71**	**-2.92, -0.49**
	P for trend			**0.001**		**0.002**
With ROA	0	-1.10	Ref.		Ref.	
	1-2	-1.19	-0.09	-1.29, 1.11	0.18	-0.99, 1.36
	3-4	-1.38	-0.28	-1.45, 0.89	-0.02	-1.19, 1.15
	5-7	-1.73	-0.63	-1.79, 0.53	-0.16	-1.39, 1.06
	P for trend			0.201		0.635

Bold denotes statistically significant result. β regression coefficient; CI confidence interval; ROA radiographic osteoarthritis; Ref reference group;

†Adjusted for age, sex, body mass index, physical activity, any pain medication, baseline lateral cartilage volume, cartilage defects and bone marrow lesions.

Table 8-3 Association between the number of painful sites and total tibiofemoral cartilage volume loss, by ROA status

	No. of pain sites	Annual percentage cartilage volume loss (mean, %)	Univariable		Multivariable†	
			β	95% CI	β	95% CI
Without ROA						
	0	-0.34	Ref.		Ref.	
	1-2	-0.97	-0.63	-1.70, 0.43	-0.82	-1.94, 0.30
	3-4	-1.97	**-1.63**	**-2.76, -0.51**	**-1.66**	**-2.83, -0.50**
	5-7	-1.76	**-1.42**	**-2.52, -0.32**	**-1.69**	**-2.90, -0.48**
	P for trend			**0.003**		**0.002**
With ROA						
	0	-1.48	Ref.		Ref.	
	1-2	-1.45	0.04	-1.13, 1.20	0.27	-0.87, 1.41
	3-4	-1.52	-0.03	-1.16, 1.11	0.33	-0.80, 1.47
	5-7	-2.08	-0.60	-1.73, 0.53	0.02	-1.16, 1.20
	P for trend			0.204		0.930

Bold denotes statistically significant result. β regression coefficient; CI confidence interval; ROA radiographic osteoarthritis; Ref reference group;

†Adjusted for age, sex, body mass index, physical activity, any pain medication, baseline cartilage volume, cartilage defects and bone marrow lesions.

Further analyses using linear regression on the association between pain at each specific site and total tibiofemoral cartilage volume loss to explore whether weight bearing or systemic factors underlies the associations. As shown in Table 8-4, hand, shoulder and back pain showed statistically significant associations with cartilage volume loss in those without (but not with) ROA after adjustment for confounders, but pain at all lower limb sites was not associated with cartilage volume loss. The significant associations remained after adjusting for multiple testing (Table 8-4). Consistent results were found after further adjustment for knee injury and common comorbidities including diabetes, heart problems, hypertension, and rheumatoid arthritis, and even mutual adjustment for pain at other sites as well as after re-analyses of data using inverse probability weighting method (Appendices 8-1 and Appendices 8-2).

Table 8-4 Association between pain at each site and total tibiofemoral cartilage volume loss, by ROA status

	Pain	Univariable		Multivariable†	
	site	β	95% CI	β	95% CI
Without ROA					
	Neck	-0.57	-1.22, 0.07	-0.67	-1.37, 0.03
	Hand	**-0.83**	**-1.47, -0.19**‡	**-0.83**	**-1.52, -0.13**‡
	Shoulder	**-0.78**	**-1.42, -0.14**‡	**-0.81**	**-1.50, -0.13**‡
	Back	-0.52	-1.17, 0.13	**-0.83**	**-1.50, -0.17**‡
	Knee	-0.54	-1.20, 0.13	-0.55	-1.22, 0.12
	Hip	-0.30	-0.95, 0.34	-0.47	-1.14, 0.20
	Foot	-0.50	-1.16, 0.16	-0.22	-0.92, 0.47
	Upper limb	**-0.38**	**-0.62, -0.14**‡	**-0.47**	**-0.72, -0.21**‡
	Lower limb	-0.28	-0.58, 0.02	-0.27	-0.58, 0.04
With ROA					
	Neck	-0.37	-1.08, 0.33	-0.09	-0.78, 0.60
	Hand	0.00	-0.71, 0.71	0.17	-0.53, 0.87
	Shoulder	-0.59	-1.33, 0.14	-0.43	-1.15, 0.29
	Back	-0.52	-1.24, 0.21	-0.22	-0.96, 0.52
	Hip	0.10	-0.61, 0.82	0.28	-0.46, 1.02
	Knee	-0.37	-1.07, 0.33	-0.02	-0.74, 0.71
	Foot	-0.05	-0.76, 0.67	0.23	-0.50, 0.95
	Upper limb	-0.18	-0.43, 0.07	-0.07	-0.33, 0.19
	Lower limb	-0.09	-0.43, 0.25	0.10	-0.27, 0.48

Bold denotes statistically significant result. β regression coefficient; CI confidence interval; ROA radiographic osteoarthritis; Ref reference group;

†Adjusted for age, sex, body mass index, physical activity, any pain medication, baseline cartilage volume, cartilage defects and bone marrow lesions;

‡Denotes significant association that passes Hochberg adjustment for multiple testing.

Appendices 8-1 Association of the number of pain sites with lateral and total tibiofemoral cartilage volume loss in those without ROA

	No. of pain sites	Multivariable†		Multivariable‡	
		β	95% CI	β	95% CI
Lateral tibiofemoral cartilage volume loss					
	0	Ref.		Ref.	
	1-2	-0.73	-1.84, 0.39	-0.72	-1.85, 0.41
	3-4	**-1.37**	**-2.52, -0.22**	**-1.23**	**-2.43, -0.03**
	5-7	**-1.71**	**-2.92, -0.49**	**-1.71**	**-2.95, -0.46**
	P for trend		**0.002**		**0.003**
Total tibiofemoral cartilage volume loss	0	Ref.		Ref.	
	1-2	-0.82	-1.94, 0.30	-0.81	-1.94, 0.32
	3-4	**-1.66**	**-2.83, -0.50**	**-1.55**	**-2.76, -0.33**
	5-7	**-1.69**	**-2.90, -0.48**	**-1.67**	**-2.91, -0.43**
	P for trend		**0.002**		**0.003**

Bold denotes statistically significant result. β regression coefficient; CI confidence interval; ROA radiographic osteoarthritis; Ref reference group;

†Adjusted for age, sex, body mass index, physical activity, any pain medication, baseline cartilage volume, cartilage defects and bone marrow lesions;

‡ Further adjusted for knee injury, diabetes, heart problems, hypertension, and rheumatoid arthritis.

Appendices 8-2 Association between pain at each site and total tibiofemoral cartilage volume loss, by ROA status

	Pain site	Multivariable†		Multivariable‡	
		β	95% CI	β	95% CI
Without ROA					
	Neck	-0.67	-1.37, 0.03	-0.61	-1.33, 0.10
	Hand	**-0.83**	**-1.52, -0.13**	**-0.74**	**-1.43, -0.06**
	Shoulder	**-0.81**	**-1.50, -0.13**	**-0.73**	**-1.42, -0.04**
	Back	**-0.83**	**-1.50, -0.17**	**-0.75**	**-1.42, -0.09**
	Knee	-0.55	-1.22, 0.12	-0.40	-1.07, 0.28
	Hip	-0.47	-1.14, 0.20	-0.30	-0.97, 0.37
	Foot	-0.22	-0.92, 0.47	-0.15	-0.83, 0.54
	Upper limb	**-0.47**	**-0.72, -0.21**	**-0.43**	**-0.77, -0.09**
	Lower limb	-0.27	-0.58, 0.04	-0.16	-0.41, 0.09
With ROA					
	Neck	-0.09	-0.78, 0.60	-0.20	-0.93, 0.53
	Hand	0.17	-0.53, 0.87	0.08	-0.65, 0.81
	Shoulder	-0.43	-1.15, 0.29	-0.56	-1.30, 0.19
	Back	-0.22	-0.96, 0.52	-0.38	-1.17, 0.41
	Hip	0.28	-0.46, 1.02	0.25	-0.51, 1.01
	Knee	-0.02	-0.74, 0.71	-0.11	-0.87, 0.65
	Foot	0.23	-0.50, 0.95	0.23	-0.50, 0.96
	Upper limb	-0.07	-0.33, 0.19	-0.19	-0.55, 0.16
	Lower limb	0.10	-0.27, 0.48	0.10	-0.23, 0.43

Bold denotes statistically significant result. β regression coefficient; CI confidence interval; ROA radiographic osteoarthritis; Ref reference group;

†Adjusted for age, sex, body mass index, physical activity, any pain medication, baseline cartilage volume, cartilage defects and bone marrow lesions;

‡Further adjusted for pain at other sites.

8.4 Discussion

This longitudinal study shows that greater number of painful sites is associated with knee cartilage volume loss, especially in those without ROA. These relationships persisted after adjustment for age, sex, BMI, physical activity, pain medication, and knee structural abnormalities at the lateral and total tibiofemoral compartments, suggesting that MSP may be an early marker of more rapid knee cartilage loss. The underlying mechanisms for this association are uncertain, but could include systemic, central or genetic factors. To our knowledge, this is the first to investigate the prospective relationship between MSP and cartilage volume loss.

The high prevalence of MSP (>2 sites) found in this study is similar to reported prevalence in previous studies [311, 315, 332, 361], despite differences in the methods of assessing pain and the number of pain sites. This finding corroborates the evidence that MSP is very common in the older general population. Consistent with some previous studies (but not all) [245, 255], knee pain was not found to be associated with high rates of cartilage volume loss in any compartments, whereas these findings differ from some of previous studies which identified the relationship between knee pain and cartilage volume loss [252, 349-351]. These discordances could be attributed to the differences in characteristics of population included, follow-up time period, and measurement and/or definition of knee pain.

The present study found that increasing number of painful sites is associated with greater cartilage volume loss. One possible explanation for this link is the involvement of systemic inflammation which have been shown to be a critical contribution to both pain [355] and OA pathogenesis [362]. In the process of inflammation, inflammatory factors are released, such as cytokines, chemokines, prostanoids, proteolytic enzymes and nerve and vascular growth factors, which can activate peripheral nociceptors, thereby leading to peripheral sensitisation and hyperexcitability of dorsal horn transmission neurons in the central nervous system (central sensitisation) [95, 363]. These inflammatory factors are also associated with increased cartilage turnover and matrix degradation [364]. Furthermore, heightened pain sensitivity can contribute to increased level of inflammatory factors releases, thus creating a cycle of inflammation, high pain sensitivity and pain severity [353]. Based upon this evidence, it is plausible that people reporting pain at greater numbers of painful sites are more likely to have a higher level of systemic inflammation, leading to more loss of cartilage volume.

Overweight or obesity is widely considered as an important risk factor for developing pain [95, 365]. Potential mechanisms include increased physical loading as well as systemic inflammation. Increasingly, evidence supports a more important role for systemic inflammation rather than physical loading, as the mechanical effect of overloading is insufficient to explaining pain at non-weight bearing joint, such as hand pain [341]. Those with greater numbers of painful sites were heavier in this study, supporting this hypothesis. However, the relationship between number of painful sites and knee cartilage volume loss did not change after adjustment for BMI, suggesting that this relationship is independent of BMI. Moreover, we found that cartilage volume loss is associated with upper limb pain rather than lower limb pain. This further supports that it is not simply due to loading.

Previous studies demonstrate a considerable genetic influence on pain. Genetic components can account for approximately 50% heritability estimates for different pain traits in twin studies [276]. Candidate gene studies have identified multiple genes associated with pain sensitivity [278-280]. Furthermore, evidence suggests that peripheral/central nervous system sensitisation and multiple biologic/psychological processes are shared pain mechanisms in multiple conditions including back pain, neck pain, shoulder pain, OA and CWP, etc [366, 367] and are strongly affected by underlying genetic factors [341]. A recent twin study also demonstrates a single underlying genetic factor which can explain pain reporting at different sites [368], and Malkin *et al.* reports that back pain and CWP are linked by shared genetic factors [369]. This indicates that back pain/upper limb pain may be a group with CWP, and could explain why back pain/upper limb pain are more predictive of cartilage volume loss. In the current study, we found that cartilage volume loss is greater in those with pain at multiple sites. It is likely that MSP in older people represents generalised OA which has a strong genetic component implicated in its' pathogenesis [370]. Therefore, combined with the finding that cartilage volume loss is greater in those with MSP, these studies imply that genetic factors may have a crucial role in determining the additive effects of MSP. Nonetheless, it is currently unknown which genes explain a larger proportion of the susceptibility to pain or cartilage loss, and whether genes linked to cartilage loss have any role in the regulation of pain, although several articular cartilage related genes were found to be associated with characterised pain phenotypes [371].

The present study failed to detect any significant association between cartilage volume loss and MSP in people with ROA, which could be explained by the stage of disease. Radiographically evident changes represent later stage OA, and therefore individuals with ROA would lose cartilage volume faster than those without ROA [372]. This is supported by our finding that people without ROA have more cartilage volume at baseline (data not shown). Conceivably, this finding may not only have great practical implications for early diagnosis, but also highlights the importance of treatment targeted at MSP as it might have beneficial effects to limit progression to end-stage disease. Also, our results showed a tendency towards increased cartilage volume loss at the medial tibiofemoral compartment, although it did not show a statistically significant association. The reason for this is unclear. This could be attributable to more mechanical effects on medial compartment, subsequently, contributing to less medial tibiofemoral cartilage volume at baseline compared with lateral compartment, so there were not too much room to move on the scale theoretically.

The strengths of the current study are the longitudinal study design, the relatively large sample size and the objectively measured outcome (cartilage volume loss, as measured by MRI). Some potential limitations in this study have to be considered when interpreting these results. These include the self-reported binary nature of the assessments of pain [95], which did not allow investigation of any effect of pain intensity on cartilage volume loss; therefore, to what extent more severe pain is associated with cartilage volume loss is still unclear. Furthermore, pain may result from other musculoskeletal diseases; however, we have not screened for these conditions. Second, we do not have images of other sites to know whether they have any site pathology, which might explain localized pain. Lastly, a possible problem with this study was loss to follow-up which may bias our results as people with more painful sites were more likely to have lower physical function which may underestimate the association. Also, for the current study, repeat MRI scans at 2.6 years were only available in a subset of the TASOAC study; however, there were no significant differences between the participants included in this study and the rest of the cohort in terms of in demographics, physical activity, ROA and the number of painful sites, apart from a higher prevalence of medial tibiofemoral cartilage defects in those not included, and the results did not alter after using inverse probability weighting, indicating that our results are robust.

In conclusion, the presence of MSP independently predicts knee cartilage volume loss, especially in people without knee OA, suggesting that widespread pain may be an early marker of more rapid knee cartilage loss in those without ROA. The underlying mechanism is unclear, but it is independent of anthropometrics, physical activity and knee structural abnormalities, possibly mediated by systemic, central or genetic factors. More research is needed to demonstrate the non-conventional role of pain at other sites in early stage of knee OA, thereby possibly facilitating the development of disease-modifying interventions.

Chapter 9: Summary, implication and future directions

9.1 Summary

As mentioned above, OA is the most common form of arthritis [60]. It is a leading cause of pain and disability accompanied by functional limitation and reduced quality of life. OA is predicted to become more prevalent as expected lifespan and rate of obesity increase, resulting in an even larger personal, social and economic burden [2]. Despite this, currently, there is no cure for OA and treatment mostly focuses on pain relief and functional improvement for the affected joints [109, 373]. Therefore, the development of prevention strategies appears to be of particular importance in the prevention of OA [374]. However, there are some obstacles in current OA research and clinical strategies which primarily utilise radiography to study, diagnose and treat the disease. This evaluation most likely reflects later disease with irreversible structural changes, which might be a key reason to explain why few interventions have been shown to be effective in both prevention and treatment; we, therefore, need to focus on an earlier stage of the disease when there are reversible structural changes [57]. This will enable us to identify risk factors or biomarkers for early stages of the disease and thus help prevent and understand the pathogenesis of how risk factors confer the development and progression of OA and pain it causes. OA is a highly heterogeneous disease with a considerable number of risk factors interacting with each other. Two important risk factors, which may have a crucial role in the identification of ‘at risk’ patients and the management of OA, are genetic factors and obesity. This thesis has investigated the role of genetic factors and/or obesity in the early structural changes on MRI and pain, with some novel and important findings, as summarised below.

Chapter 4 examined the associations between family history of knee OA and knee structural changes over 8-10 years. Offspring having at least one parent with TKR for severe primary knee OA were found to have a greater increase in cartilage defects, meniscal extrusion and tears in the medial tibiofemoral but not lateral compartment than controls with no family history of knee OA. BMLs were not different between offspring and controls. Furthermore, family history of knee OA was associated with increased risk of worsening cartilage defects, meniscal extrusion and tears before and after adjustment for potential confounders, suggesting that the effects of genetic factors are pleiotropic being associated with the progression of multiple structural abnormalities, and thus supporting the notion that genetic factors may influence the tissue of entire joint other than cartilage. These associations were only seen at the medial compartment suggesting site-specific effects of genetic factors. There was no significant association for BMLs, suggesting a greater environmental effect influence changes in BMLs; which implies they may be modifiable. Although multiple environmental factors have been adjusted in this study, we were unable to rule out and calculate the weight of environmental factors in the progression of cartilage defects and meniscal pathology. Whilst cartilage preservation and repair have become realistic, recent developments in the tissue engineering research, in particular stem-cell-based therapy, have achieved functional replacement of articular cartilage, implying that cartilage damage is modifiable despite genetic control [375].

Chapter 5 explored the associations of weight with knee cartilage volume/defects over 10 years in offspring and controls. This long-term follow-up study extends prior observations and provides a strong support for the deleterious effects of increasing weight on the cartilage damage and loss. Increasing weight was negatively associated with cartilage volume and the presence of cartilage defects in offspring, but no significant associations were found in controls. Interestingly, the effect sizes of weight on both cartilage defects and volume were significantly and consistently higher than that in controls. This most likely indicates gene-environment interaction with regard to overweight/obesity in the pathogenesis of early stage of the disease. Our study also reflects a role of age mediating these associations based on the findings of a significant interaction between time and weight on lateral cartilage volume, suggesting aging makes the knee joint more susceptible to the effects of weight. Although the association between weight and lateral cartilage volume did not reach statistical significance in offspring with an adverse effect of weight gain on cartilage volume, a significant interaction between weight and time on lateral cartilage volume indicates that a longer time-frame follow-up may be needed to detect a significant association in this relatively younger population.

Chapter 6 described the prevalence of knee pain and its change between offspring and controls, and examined the associations between family history of knee OA and change in knee pain measured by WOMAC over 8 years. Knee pain was more prevalent in offspring than in controls at baseline and 10 years. Also, offspring had higher scores in total pain and each subscale at both 2 years and 10 years, although some scores at 2 years were not statistically significant. In relative to controls, greater changes in pain scores and a high proportion of worsening knee pain (≥1) were observed in offspring. Offspring were found to have about two-fold higher risk of worsening total knee pain and each subscale, and these associations were independent of structural abnormalities and potential confounders, implying that genetic components may be implicated in the pathogenesis of pain, and the genesis of pain may result from factors outside the joint possibly attributable to difference in pain processing.

Chapter 7 described the prevalence of MSP and investigated the longitudinal associations of fat mass, FMI and BMI with MSP as well as site-specific pain over 5.1 years in a population-based study of older adults. This study further corroborates that MSP is extremely prevalent in community-based older population. This is the first study to show longitudinal associations between fat mass and MSP, finding that fat mass was associated with MSP and this association was independent of demographic factors, physical activity, psychological health, education level and employment. This suggests an important role of fat mass in the pathogenesis of MSP. Fat mass, FMI and BMI were found not only to associate with pain at weight-bearing sites (hips, knees and feet), but with hand pain, implying that the underlying mechanisms by which obesity contributes to MSP may be due to both physical loading and systemic inflammatory factors, but the latter one appears to be more important as systemic inflammation has been recognised as an important factor in driving the development of peripheral and central sensitisation, thereby leading to pain at multiple sites. Given that MSP may reflect high systemic inflammation and pain processing; this prompted the work done in **Chapter 8** investigating the association between MSP and the hallmark of knee OA (cartilage volume loss).

Chapter 8 examined whether pain at multiple sites predicts knee cartilage volume loss over 2.6 years and explored the potential mechanisms. There was a tendency towards an increase of cartilage volume loss in medial, lateral and total tibiofemoral compartments as number of painful site increased. These relationships persisted for lateral and total tibiofemoral cartilage volume loss after adjustment for potential confounders suggesting that MSP is associated with more cartilage volume loss. Notably, these positive and dose-response relationships between number of painful sites and cartilage volume loss were only seen for those without radiographic OA, this highlights the predictive role of number of painful sites in the early pathogenesis of knee OA. The potential mechanism mediating these relationships more likely involve systemic inflammation because of the findings that cartilage volume loss was associated with upper limb pain (hand, shoulder and back), but not pain at lower limb sites as well as other mechanisms such as central and genetic factors.

To sum up, this series of related analyses based on two prospective studies of younger and older population shed light on the role of genetic and systemic inflammatory factors in the pathogenesis of OA and pain. These results not only highlight the possibility of identifying ‘at risk’ patients earlier, but, most importantly, offer an opportunity to develop therapeutic approaches targeting those at high risk of the disease and its symptom.

9.2 Implication and Future directions

There are some very novel findings from this thesis based on two prospective studies of younger and older population which have great significance for the development of prevention and treatment strategies for OA and its symptoms. **Chapter 4** and **Chapter 6** examined the role of family history of knee OA in the progression of early knee structural changes and knee pain, suggesting a possible role of genetic components in the early stage of the disease and pain. Currently, phenotype standardisation remains a question to be solved in the genetic studies of OA, although a more homogenous phenotype for OA has been examined in which the cases were defined as TJR only [376]. It is hoped that genetic studies focus on endophenotypes that are measureable, underlying and intermediate biomarkers with a closer and direct relationships to the disease may be of particular advantage in such a heterogeneous condition [377]. As with other complex diseases, endophenotypes in OA are attracting increased attentions to unravel genetic contribution to OA [131]. An example in OA is the use of joint space width on radiographs as a surrogate for cartilage thickness in which DOTL1 locus was identified associated with cartilage thickness [378, 379]. Cartilage defects and meniscal pathology have been associated with knee OA [26, 380], and our results have shown that these structures are under genetic control. Given these, these early structures could be promising and ideal endophenotypes to be studied in the future OA genetic studies [133]. In contrast, progression in BMLs is more likely to be affected by environmental factors, suggesting that BMLs may be more amenable to modification. This is supported by a clinical trial from our group that found use of a single dose of zoledronic acid (5 mg) can significantly reduce BML size compared to placebo after six months [381]. The influence of genetic factors on knee pain also found in the **Chapter 6** suggests that pain may represent a crucial phenotype related to OA to study, but limited studies are available to date with only one GWAS which identified genes to link CWP [177]. Identification of risk loci implicated in the pathological process of OA and pain may be clinically beneficial for the development of novel therapeutic approaches.

Individuals with family history of OA appear to develop the disease and progress earlier in the **Chapter 4**. This implies that those with OA family history may be of great utility in the development of biomarker of early OA and the design of clinical trials to maximize the differentiation of interventions and placebo groups. **Chapter 6** showed that the associations between family history of OA and worsening knee pain are independent of structural abnormalities, suggesting that structural lesions may not be major elements for developing and maintaining pain, possibly due to the involvement of peripheral and central nervous system sensitization. It is therefore suggested that the limitations of use of analgesics targeting peripheral knee joint alone in the clinical setting and structural modification may not be sufficient in the management of OA-related pain. Agents with central action targeting pain mechanisms are needed to be further developed and tested in future studies. Also, future studies on why the correlation between structures and pain is poor are warranted, for example, studies using brain positron emission tomography (PET) and functional MRI may facilitate our understanding about importance of alterations in brain functional connectivity, affective and motivational aspects of pain, and how systemic inflammation induced by tissue damage (peripheral stimuli) leads to the development of central sensitization [382].

Chapter 5 demonstrated greater effect sizes for the associations between weight and knee cartilage loss and defects in the offspring than that in controls, suggesting gene-environment interaction with regard to obesity in the pathogenesis of knee OA. Our results highlight that achievement and maintenance of a healthy weight are of great importance to avoid early stage of the disease, particularly in those with family history of OA. There is evidence to suggest that modest weight loss in early stage of disease process appears to be a more effective method in decreasing the incidence and progression of symptomatic OA than in the later stage of the disease [383]. Therefore, early screening of family history combined with an important risk factor (obesity) could be the most effective way for identifying individuals at high risk and open up an opportunity to prevent and treat the disease earlier. For instance, consideration should be given in monitoring weight in obese individuals if there is a positive OA family history. Those individuals at high risk should be encouraged to avoid weight gain through participation in physical activity, healthy dietary intake. Bariatric surgery is the most effective treatment for weight loss in the short-term [384]; therefore, surgical treatments may significantly reduce the risk of OA in obese individuals with family history, this could be trialed. Furthermore, the impact of weight loss and the efficacy of disease modifying OA drug on knee structures warrant further investigation in the subgroup of individuals most at high-risk. Taken together, the findings from **Chapter 4, 5 and 6** that environmental factors (obesity) interact with genetic factors to increase the risk of early structural changes and symptoms, and BMLs are more likely to be affected by environmental factors highlight the importance of measurement of inflammatory marker (such as CRP) in this genetically loaded cohort. Future research in this field is needed, and thus allow to develop effective prevention and intervention strategies.

Not only does weight loss have a beneficial effect on the prevention of early stage of OA, but is beneficial for pain management. **Chapter 7** found that fat mass was associated with MSP, lower limb and hand pain, suggesting that other than physical loading, systemic inflammatory factors may have an important role in the pathogenesis of pain. Hence, weight management and maintenance should be emphasized across population groups to achieve a public health goal. Exercise and healthy diet should be advocated; however, which approaches can effectively achieve weight loss and maintain weight remains challenging. Systemic inflammatory factors present the potential target for intervention, so treatments aimed at reducing the level of inflammation and blocking biosynthesis of adiposity may have a role in alleviating pain. Further studies are needed to investigate which mechanisms (physical loading or systemic inflammation) play more important role in the pathogenesis of pain and which inflammatory markers directly participate in pain generation.

Chapter 8 supports a role of systemic factors in the cartilage volume loss. Number of painful sites was associated with cartilage volume loss over 2.6 years before and after adjusting for possible confounding factors, suggesting that there may be a role of systemic inflammation as a mediator of this effect affecting the progression of knee OA. A systematic review from our group have concluded that serum CRP is significantly associated with symptoms rather than with ROA [385]. Also Haugen *et al.* [386] found that symptomatic hand OA, but not radiographic hand OA is related to elevated risk of coronary heart disease events, indicating pain may be a marker of systemic inflammation. There have been conflicting results regarding the relationship between knee pain alone and knee cartilage volume loss; surprisingly, a dose-response relationship between number of painful sites and cartilage volume loss was observed. Given this, those with greater number of painful sites may have a higher level of systemic inflammation, and other than affected joint, pain at other sites should be taken into account in the OA research and clinical trial. Interestingly, the associations of number of painful sites and cartilage volume loss were stronger in those without radiographic knee OA than with radiographic knee OA. This, therefore, not only presents the possible opportunity for the early diagnosis of knee OA through simply counting the number of pain sites, but may allow for the development of therapeutic strategies through targeting MSP with anti-inflammatory and centrallyacted drugs, which would be more relevant to the treatment of progression of OA. Despite no clinical trial investigating this directly, several previous studies seem to support this. A multicentre clinical trial [387] shows that licofelone, an analgesic and an anti-inflammatory, can significantly reduce cartilage volume loss over time in patients with knee OA. Moreover, it has been reported that long-term use of NSAIDs was associated with less changes in joint space width values compared to nonusers [388]. It is, therefore, possible that treating MSP through anti-inflammatory pathway may lead to a reduction of the progression of OA, and further clinical trials on MSP are required.

In conclusion, these analyses of data from two prospective cohort studies have demonstrated that genetic effects, environmental effects and their interactions with each other play an important role in the pathogenesis of early stage of knee OA and its symptoms (pain). The findings from this thesis may enable the early identification of high-risk groups, and allow for the identification of novel treatment strategies of early stage of the disease. Future research is needed to investigate screening family history of OA in the prevention of OA and to better define a definite relationship between systemic inflammation and pain in OA.

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